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(54) Title: KCNQ POTASSIUM CHANNELS AND METHODS OF MODULATING SAME

(57) Abstract

The present invention relates to KCNQ proteins defining potassium channels. In particular, the invention concerns the human KCNQ2, human KCNQ3, murine KCNQ2, and rat KCNQ2 proteins reported herein. KCNQ2 and KCNQ3 proteins are nervous system–selective and may be involved in neurotransmission and neuroprotection. The KCNQ2 and KCNQ3 of the present invention can be used to assay for modulators of the proteins, which would be useful in treatment of such disorders as ataxia, myokymia, seizures, Alzheimer's disease, Parkinson's disease, age–associated memory loss, learning deficiencies, motor neuron diseases, epilepsy, stroke, and the like.

KCNQ POTASSIUM CHANNELS AND METHODS OF MODULATING SAME

Field of the Invention

The present invention concerns nucleic acids and proteins for potassium i channels, as well as related vectors, host cells, processes for preparation, and meethods

5 of use. Included within the present invention are methods of screening for comppounds that bind to and/or otherwise modulate the potassium channel proteins disclosedd herein. Additionally, the present invention encompasses methods of modulatingg the potassium channels disclosed herein, for example methods of opening/activatingg or closing/inactivating said potassium channels.

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Background of the Invention

Among ion channels, potassium ion (K^+) channels are the most ubiquitouus and diverse. They include three major structural classes - channels with six, four, orr two transmembrane domains. The six transmembrane domain potassium channels aare

15 divided further into different families, such as Shaker-like, eag-like and Slo-likee potassium channels. Recent identification of KvLQT1 established a new familyy of six-transmembrane potassium channels. Barhanin et al. (1996) Nature 384: 78-880; Sanguinetti et al. (1996) Nature 384: 80-83; Yang et al. (1997) Proc. Natl. Acad.l. Sci. USA 94: 4017-22; Wang et al. (1996) Nature Genetics 12: 17-23. Search of DNNA and

20 protein sequence databanks reveals additional potential members of KvLQT1-reelated channels in C. elegans as well as in the human. Wei et al. (1996), Neuropharmacology 35: 805-29; ; Yang et al. (1997) Proc. Natl. Acad. Sci. USAA 94: 4017-2.

One or more types of K^+ channels reside on cell membrranes

25 where they are remarkably selective for K^+ over other ions. In excitable cells, K^{++} channels modulate action potential configuration. Efflux of potassium is the maajor mechanism for repolarization, maintenance, and hyperpolarization of resting membrane potential. Halliwell (1990) in Potassium channels-structure, classificcation, function and therapeutic potential (N. S. Cook, ed.); 348-381; Jan, L. Y. and Jann, Y.

30 N. (1992), Ann. Rev. Physiol. 54: 537-55; Pongs (1992), Physiol. Rev. 72: S69-;-S88.

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In neurons, K^+ channels regulate neuronal excitability, action potential shaape and firing pattern, and neurotransmitter release. These channels can be gated by various stimuli, such as intracellular second messengers, membrane potential, ionns, and neurotransmitters. Hille (1992), <u>Ionic channels of excitable membranes</u>; Catttterall

5　　(1995), <u>Ann. Rev. Biochem.</u> 64: 493-531. Neuronal K^+ channels are critical to suuch neuronal functions as neurotransmission and neuroprotection, and they may affecct perception, learning, behavior, and the like.

Recently, the nomenclature for KvLQT1 and the KvLQT1-related channeels was changed. Biervert et al. (1998), <u>Science</u> 279:403-406. KvLQT1 was re-namned

10　　KCNQ1, and the KvLQT1-related channels (KvLR1 and KvLR2) were re-namedd as KCNQ2 and KCNQ3, respectively. Therefore, throughout this specification, reference to KCNQ1 is equivalent to KvLQT1; reference to KCNQ2 is equivalennt to KvLR1; and reference to KCNQ3 is equivalent to KvLR2.

Benign familial neonatal convulsions ("BFNC"), a class of idiopathic

15　　generalized epilepsy, is an autosomal-dominantly inherited disorder of newborns.3. BFNC has recently been linked to mutations in two putative K^+ channel genes, KCNQ2 and KCNQ3. Biervert et al., <u>supra</u>; Charlier et al. (1998), <u>Nature Genettics</u> 18:53-55; Singh et al. (1998) <u>Nature Genetics</u> 18:25-29. Preliminary functional I characterization of KCNQ2 confirmed that this gene encodes a voltage-activated I K^+

20　　channel. Singh et al., <u>supra</u>.

Summary of the Invention

The present invention discloses novel nervous system-specific potassium ι channels referred to herein as KCNQ2 (formerly called KvLR1) and KCNQ3

25　　(formerly called KvLR2). Within the present invention are human KCNQ2 (Figuure 2), human KCNQ3 (Figure 23), murine KCNQ2 (Figure 10), and rat KCNQ2 (Figgure 16 and Figure 17). The invention encompasses the amino acid sequences of thesse proteins and the nucleic acid sequences encoding said proteins, as well as variatioons in the nucleic acid sequences due to degeneracy in the genetic code.

The present invention provides for nucleic acid molecules at least about 770% identical to the consensus sequence of the nucleotide sequences disclosed herein.ı. Preferably, the present invention provides: (a) a purified and isolated nucleic aciad molecule encoding a KCNQ2 and/or KCNQ3 protein of the present invention; (bb)

5   nucleic acid sequences complementary to (a); (c) nucleic acid sequences having ɛ at least 70% sequence identity, more preferably at least 80%, more preferably at leaast 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) which will hybridize to (a) or (b) unnder stringent conditions, said fragment preferably comprising at least 15 nucleotides.ı.

10   Preferred nucleic acid sequences encoding the KCNQ2 and KCNQ3 proteins of t the present invention are found in SEQ ID NO:3, SEQ ID NO:17, SEQ ID NO:7 andd SEQ ID NO:5.

Also within the scope of the present invention are amino acid sequences ɛ at least about 70% identical to the consensus sequence of the proteins disclosed herrein.

15   Preferably, the invention covers: (a) amino acid sequences comprising the KCNCQ2 and/or KCNQ3 proteins of the present invention; and (b) amino acid sequences hhaving at least 70% sequence identity, more preferably at least 80%, more preferably at ! least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a). Preferred amino acid sequences comprising the KCNQ2 and KCNQ3

20   proteins of the present invention are found in SEQ ID NO:4, SEQ ID NO:18, SEEQ ID NO:8 and SEQ ID NO:6.

The invention further concerns novel nucleic acids and associated vectorss, host cells, and methods of use. Preferably, the nucleic acid molecule is a DNA moleccule. Further preferred are nucleotide sequences encoding the amino acid sequences oьf SEQ

25   ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:18 as wwell as proteins about 70% or more identical to these sequences. Also preferred are nucleotide sequences about 80% or more identical to SEQ ID NO:1; most preferrred are SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:17.

The invention further concerns nucleic acids obtained by PCR with degernerate

30   oligonucleotide primers. Persons of ordinary skill in the art could devise such pnrimers

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based on the consensus sequence described herein. PCR techniques are describeed in White et al. (1989), Trends Genet. 5: 185-9.

This invention further concerns nucleic acid vectors comprising a nucleicc acid sequence coding for a KvLR/KCNQ protein, host cells containing such vectors, ; and

5   polypeptides comprising the amino acid sequence of a KvLR/KCNQ protein. Preferably, the vector encodes a full-length KvLR/KCNQ protein and the polypeeptide is full-length KvLR/KCNQ protein. The inventors prefer frog expression vectorrs such as pSP64T or derivatives thereof (Melton et al. (1984), Nucl. Acids Res. 12: 70557-70); mammalian cell expression vectors such as pcDNA3 (available from Invitroogen);

10   or bacterial cell expression vectors such as pET-30 (available from Novagen or Promega).

This invention further concerns host cells transformed with the above-described vectors. The inventors prefer Xenopus oocytes, mammalian cells (e.g.5., HEK293, CHO, L929), and bacterial cells (e.g., E. coli, especially BL21(DE3),

15   available from Novagen). The inventors particularly prefer the cells deposited aas ATCC Acc. No. CRL-1573 (American Type Culture Collection, 10801 Universiiity Boulevard, Manassas VA 20110-2209).

The invention also concerns methods for detecting nucleic acids coding ffor KCNQ/KvLR proteins and processes for detecting molecules that bind to and/or r

20   otherwise modulate the activity of KCNQ/KvLR proteins. "Modulate" encompaasses both channel openers/activators and channel closers/inactivators.

The invention also concerns methods of modulating the KCNQ proteins, , specifically methods of opening/activating or closing/inactivating the KCNQ2 annd/or KCNQ3 channels. Additionally, the present invention encompasses a method obf

25   treating disease by modulating the activity of the KCNQ proteins. .

All references cited herein, whether *supra* or *infra*, are hereby incorporatted herein in their entirety.

Description of the Drawings

30   Figure 1 shows isolation of a full-length human KCNQ2/KvLR1 cDNA.. A full-length human KCNQ2/KvLR1 cDNA was derived from two overlapping cDDNA

clones. "S1" through "S6" signify transmembrane domains 1 through 6; "H5" signifies the pore-forming domain (this domain is also referred to herein as the "]'P" domain); ORF, the open-reading frame; 3' UTR, the 3' untranslated regions. Thee locations of various EST clones and probes also are shown. (The figure is not drirawn

5   to scale.)

Figures 2A and 2B show the nucleotide and deduced amino acid sequencce of human KCNQ2/KvLR1.

Figure 3 shows a sequence comparison of human KCNQ2/KvLR1 and huuman KCNQ1/KvLQT1. "|" denotes amino acid sequence identity. The C-terminal amnino

10   acids of both proteins are not shown.

Figure 4 shows a sequence comparison of human KCNQ3/KvLR2 and huuman KCNQ1/KvLQT1. "|" denotes amino acid sequence identity. The C-terminal amnino acids of both proteins are not shown. The N terminal amino acids of KCNQ3/KívLR2 are not shown.

15   Figure 5 shows expression of KCNQ2/KvLR1 and KCNQ3/KvLR2 in huuman tissues and various portions of human brain. Figure 5A shows KCNQ2/KvLR1. . Figure 5B shows KCNQ3/KvLR2. Poly(A+) mRNA Northern blots were hybriddized individually to radiolabeled KCNQ2-specific (Figure 5A) or KCNQ3-specific (FFigure 5B) probes. RNA molecular weight markers are indicated on the left.

20   Figure 6 shows functional characterization of KCNQ2/KvLR1 currents irin Xenopus oocytes.

In Figures 6A and 6B, families of currents from water-injected (Figure 6ÁA) and human KCNQ2/KvLR1 cRNA-injected (Figure 6B) oocytes were elicited byy 1 second voltage steps, from a holding potential of -80 mV, to test potentials rangiỉing

25   from −100 to +40 mV in 10 mV increments.

Figure 6C shows the peak current-voltage (I-V) relationship for oocytes expressing human KCNQ2/KvLR1. Currents were recorded using the protocol described above for Figures 6A and 6B.

Figure 6D shows dependence of tail current reversal potential (E_{rev}) on thhe

30   external K^+ concentration. Tail currents were elicited at potentials of −110 to +1l0 mV following a 1 second pulse to +20 mV (n = 6 oocytes) while the external K^+

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concentration was varied between 2, 10, 40, and 98 mM. E_{rev} under each conditition was determined for each oocyte by measuring the zero intercept from a plot of taail current amplitude versus test potential. The dashed line has a slope of 58 mV annd is drawn according to the Nernst equation for a perfectly selective K^+ channel. Datta are

5   the mean ± SEM from six experiments.

Figure 7 shows pharmacologic characterization of KCNQ2/KvLR1 curreents in Xenopus oocytes. In particular, this figure shows effects of E-4031, 4-AP, TEA, charybdotoxin and clofilium on human KCNQ2/KvLR1 current. Superimposed l currents were recorded during 1 second steps to +30 mV, from -80 mV, during tlthe

10   same experiment. Compounds were applied via bath perfusion in order from top) to bottom. The bath was perfused with control solution for 5 minutes, or until effeccts reversed completely, between compounds. Similar results were obtained in threee additional oocytes.

Figure 8 shows co-expression of minK and human KCNQ2/KvLR1 in

15   Xenopus oocytes.

Figure 8A shows the effect of 1 mM TEA on membrane currents recordeed from an oocyte injected with human KCNQ2/KvLR1 alone. Superimposed currcents were recorded during 1 second voltage steps to +40 mV from a holding potentiahl of - 80 mV before and after applying TEA via the bath. TEA reduced human

20   KCNQ2/KvLR1 current by over 80%.

Figure 8B shows the effect of 1 mM TEA on membrane currents recordeed from an oocyte injected with minK and human KCNQ2/KvLR1. Currents were : elicited using the protocol in Figure 8A. TEA partially inhibited minK + humann KCNQ2/KvLR1 currents, however, the amplitude and kinetics of the TEA-insennsitive

25   current component were similar to currents observed in oocytes injected with miiinK alone.

Figure 9 shows murine KCNQ2/KvLR1 expression in the brain of adult mouse. Figure 9A is a dark-field photograph from a coronal section through an ¿ adult mouse brain hybridized with a radiolabeled antisense KCNQ2/KvLR1 probe, shaowing

30   KCNQ2/KvLR1 transcripts in the pyramidal cell layers of the hippocampus. Loower levels of expression were detected in the granular cell layer of the dentate gyrus.:.

Figure 9B is a dark-field photograph from a similar region as shown in Figure 9AA, but hybridized with a sense probe; little KvLR1-specific expression was detected witith this probe. Magnification: 125x for both Figure 9A and Figure 9B.

5   Figure 9C shows partial murine KCNQ2/KvLR1 nucleotide sequence (SEEQ ID NO:9) and amino acid sequence (SEQ ID NO:10). This sequence was obtained through PCR amplification of a mouse brain cDNA library using the oligonucleootides MABms 278 (SEQ ID NO:11) and MABms 315 (SEQ ID NO:12) based on the human KCNQ2/KvLR1 sequence. The PCR fragments were isolated, subclonedd, and sequenced. A 226 bp fragment as shown above was used in a probe for in situ

10   hybridization. The nucleotide sequence is 80% identical to human KCNQ2/KvLR1 (96% identity in amino acid sequence).

        MABms 278 (SEQ ID NO:11):

                5'-GGCCGAATTCTGTTTCTCAGCAGCTCCAGC-3'

        MABms 315 (SEQ ID NO:12):

15   5'-GCGCGAATTCGAGCAGCACAGGCA(A/G)AA(A/G)CA-33'

        Figure 10A through Figure 10D show the DNA and translated amino acidd sequence of the mouse brain KCNQ2/KvLR1 gene. Figure 10E shows hydropatithy analysis of the mouse brain KCNQ2/KvLR1 gene. The hydropathy plot reveals t the pattern typical of voltage-sensitive K^+ channels with 6 putative membrane spannining

20   domains (S1-S6) and a pore region (P).

        Figure 11 shows sequence alignment of the mouse heart KCNQ1/K vLQ1T1 and mouse brain KCNQ2/KvLR1 potassium channels. The alignment of these twwo genes shows a 40% overall amino acid identity (indicated by the shaded areas) annd 62.5% identity within the spanning and pore domains. Putative membrane spannning

25   and pore domains are indicated by the boxes. The signature sequence for a potasssium channel, GYG, is observed within the pore region and the voltage sensor, RXXQXXRXXR, is within the S4 domain.

        Figure 12 shows alternative splice exons in the 3' end of murine KCNQ2/KvLR1. At least two splice exons, that when translated give the amino ﹀ acid

30   sequences shown in A and B (SEQ. ID. NOS.: 13 and 14), have been identified iin the murine KCNQ2/KvLR1 gene at amino acid positions 372 and 406, respectively. .

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Figure 13 shows a mouse multiple tissue northern blot. A northern blot wwas probed with a fragment of the mouse KCNQ2/KvLR1 gene (nucleotides 1140-23806). A single transcript of 8.2 kb is observed in brain, but not is seen in other tissues.

Figure 14 shows in situ hybridization of rat brain. The composite shows t three

5   regions where the rat KCNQ2/KvLR1 message is strongly expressed. The antiseense probes show strong signal in the hippocampus, dentate gyrus, cortex, and motor nucleus of the trigeminal nerve. Sense probe controls show little background.

Figure 15 shows electrophysiology characterization of mouse KCNQ2/KvLR1-mediated whole-cell currents expressed in Xenopus oocytes. Inn

10   Figure 15A, 10 mV step depolarizations from a -80 to +40 produced a family of outward currents that were significantly different than control cells. Addition of ? 1 mM TEA blocked the KvLR1-mediated currents and background chloride currennts were not affected by TEA. Clofilium, a blocker of heart I_{Ks} and I_{Kr} currents, was ; shown to partially block the KCNQ2/KvLR1- mediated currents when depolarizeed to

15   from -80 to +40 µV. Figure 15B shows uninjected controls.

Figure 16 shows an alignment of the consensus nucleotide sequence (SECQ ID NO:1) and the nucleotide sequences of the human KCNQ3/KvLR2 (SEQ ID NO):17), human KCNQ2/KvLR1 (SEQ ID NO:3), mouse KCNQ2/KvLR1 (SEQ ID NO:55), and rat KCNQ2/KvLR1 (SEQ ID NO:7). "|" denotes sequence identity; "−"

20   represents non-consensus sequence; and "*" denotes a space introduced to optimmize sequence identity.

Figure 17 shows an alignment of the consensus amino acid sequence (SECQ ID NO:2) and the amino acid sequences of the human KCNQ3/KvLR2 (SEQ ID NOO:18), human KCNQ2/KvLR1 (SEQ ID NO:4), mouse KCNQ2/KvLR1 (SEQ ID NO:66),

25   and rat KCNQ2/KvLR1 (SEQ ID NO:8) proteins. As in Figure 16, "|" denotes sequence identity; "−" represents non-consensus sequence; and "*" denotes a spaace introduced to optimize sequence identity.

Figure 18 shows the functional characterization of KCNQ3 currents. Figgure 18A shows families of currents from KCNQ3 cRNA-injected oocytes elicited byy 1 sec

30   voltage steps, from a holding potential of -80 mV, to test potentials ranging fromn -70 to +50 mV in 10 mV increments. Figure 18B shows I-V relationship for oocytess

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expressing KCNQ3 (n=6). Currents were recorded using the protocol in Figure 118A.

Figure 18C shows dependence of tail current E_{rev} on the external K^+ concentratition.

The line has a slope predicted by the Nernst equation for a perfectly selective K^{++}

channel. Each value is the mean±SEM from 6 oocytes. Figure 18D shows effectts of

5   E-4031, 4-AP, TEA and clofilium on KCNQ3 current. Superimposed currents wvere

recorded during 1 sec steps to +20 mV, from -80 mV, during the same experimennt.

Compounds were applied via bath perfusion in order from top to bottom. The baath

was perfused with control solution for 5 min, or until effects reversed completelyy,

between compounds. Similar results were obtained in three additional oocytes.

10   Figure 19 shows co-expression of KCNQ2 and KCNQ3. Figure 19A shonws

families of currents from KCNQ2, Figure 19B from KCNQ3, and Figure 19C froom

KCNQ2+KCNQ3 cRNA-injected oocytes elicited by 1 sec voltage steps, from a ι

holding potential of -80 mV, to test potentials ranging from -70 to +50 mV (10 nmV

increments). Figure 19D shows current-voltage (I-V) relationship for oocytes

15   expressing KCNQ2+KCNQ3 (n=6). Currents were recorded using the protocol iin

Figures 19A-19C. Figure 19E shows dependence of tail current reversal potentiaal

(Erev) on the external K^+ concentration. The dashed line has a slope predicted b?y the

Nernst equation for a perfectly selective K^+ channel. Each value is the mean±SEEM

from 6 oocytes. Figure 19F shows the effects of 4-AP, TEA, charybdotoxin and ι

20   clofilium on KCNQ2+KCNQ3 current. Superimposed currents were recorded duuring

1 sec steps to +20 mV, from -80 mV, during the same experiment. Compounds vwere

applied via bath perfusion in order from top to bottom. Similar results were obtaained

in 4 additional oocytes.

          Figure 20 shows the interaction of KCNE1 (minK) with KCNQ2+KCNQ?3

25   currents. Families of currents from KCNE1 (Figure 20A), KCNQ2+KCNQ3 (Figgure

20B) and KCNQ2+KCNQ3+KCNE1 (Figure 20C) cRNA-injected oocytes elicitded by

1 sec voltage steps, from a holding potential of -80 mV, to test potentials rangingg from

-70 to +50 mV (10 mV increments). Inset of Figure 20A shows KCNE1 currentss

elicited by 5 sec voltage steps from -80 mV to potentials ranging from -30 to +500 mV

30   (20 mV increments) in the same oocyte.

Figure 21 is a photograph of in situ hybridization with rat KCNQ2 showing a cross section of the rat spinal cord. Figure 21(A) is under low magnification (55x); several areas can be visualized with a relatively high signal. Figure 21(B) is under higher magnification (322x); each high signal area is one cell and they appear by / their

5   size to be motoneurons.

Figure 22A shows macroscopic murine KCNQ2 current recorded from inside-out membrane patch excised from a CHO cell stably expressing murine KCNQ2. The current displays slow activation and outward rectification. Figure 22B shows patch clamp recording of single channel currents in an excised inside-out patch from a

10 CHO/murine KCNQ2 cell. There are at least 2 channels in the patch; single channel conductance of KCNQ2 was estimated to be between 24 and 30 pS. All recordings were made in symmetrical 140 mM K^+ using standard techniques.

Figure 23 shows the nucleotide and deduced amino acid sequence of human KCNQ3 (also referred to herein as KvLR2).

15

Detailed Description of the Invention

The following definitions apply to the terms used throughout this specification, unless otherwise defined in specific instances:

"cloning" - isolation of a particular gene from genetic material, for example a gennome,

20   genomic library, or cDNA library into a plasmid or other vector;

"KvLR protein" - a protein having at least about 70% identity with the consensuss

          sequence (SEQ. ID. NO.: 2). It may also be referred to as a "KCNQ

          protein", "KvLR/KCNQ protein" or "KCNQ/KvLR protein".

"KCNQ1" - the protein formerly known as KvLQT1.

25 "KCNQ2" - the protein formerly known as KvLR1.

"KCNQ3" - the protein formerly known as KvLR2.

"stringent conditions " (as used concerning nucleic acid hybridization)—For exanmple,

          Southern blotting washed in 1 X SSC and 0.1% SDS at a temperature cof at

          least about 42 °C. For additional stringent conditions, see Maniatis et ; al.,

30   Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboraratory,

          Cold Spring Harbor, NY (1982).

10

"multi-copy plasmid"—a plasmid having greater than one copy present in a cell

(typically 10 to 30 copies);

"Northern blotting"—a method of identifying particular RNA fragments by

hybridization with a complementary nucleic acid, typically a cDNA or r an

5   oligonucleotide;

"open reading frame" or "ORF"—a DNA sequence containing a series of nucleotitide

triplets coding for amino acids and lacking any termination codes;

"plasmid"—cytoplasmic, autonomously replicating DNA elements found in

microorganisms;

10   "promoter"—a region on DNA at which RNA polymerase binds and initiates

transcription; and

"Southern blotting"—a method of identifying particular DNA fragments by

hybridization with a complementary nucleic acid, typically a cDNA or r an

oligonucleotide.

15   For definitions of other terms in this specification, see F. Sherman et ahl.,

Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor

Laboratory, Cold Spring Harbor, NY (1987) and Lewin, B., Genes IV, Oxford

University Press, Oxford (1990).

The following definitions apply to abbreviations in this specification, unless

20   otherwise defined in specific instances:

BFNC	benign familial neonatal convulsions
BLAST	basic local alignment search tool
CHO	Chinese hamster ovary cells
DTT	dithiothreitol
DRG	dorsal root ganglion
EDTA	ethylene diamino tetraacetic acid
EST	expressed sequence tags
GPCR	G-protein–coupled receptor
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline

PCR	polymerase chain reaction
SDS	sodium dodecyl sulfate
SSC	buffer containing 150 m$\underline{M}$ NaCl, 15 m$\underline{M}$ Na₃citrate • 2 H₂O, pH 7.0.
TEA	tetraethylammonium

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For additional abbreviations, see Aldrichimica Acta, Vol. 17, No. 1 (19984).

Use and utility

It is believed by those skilled in the art that KCNQ proteins may be involved in neurotransmission. Persons of ordinary skill in the art can use KCNQ/KvLR

10 proteins of the present invention to assay for modulators of KCNQs/KvLRs. KCTNQ modulators would be useful in treatment of such disorders as ataxia, myokymia, seizures (e.g., epileptic seizures), Alzheimer's disease, Parkinson's disease, age-associated memory loss, learning deficiencies, motor neuron diseases, stroke, andd the like.

15 Because KCNQ2 and KCNQ3 are nervous system-selective potassium channels, drug specificity is built into any KCNQ2/KCNQ3-specific modulator. . A drug specific for KCNQ2 and/or KCNQ3 protein would thus avoid side-effects oon peripheral tissues that contain potassium channels. Significantly, KCNQ2/KCNCQ3-specific modulators would avoid side-effects on the heart, which contains numercous

20 types of potassium channels.

The KCNQ nucleic acids of the present invention, or antisense nucleic aci:ids, may be useful therapeutic or diagnostic agents. For such gene therapy, the nuclei:ic acids may be incorporated into vectors and/or formulated as described below and ɪ in further detail in the art.

25 Persons skilled in the art can use the polypeptides and nucleic acids of thi:is invention to prepare vectors, cells or cell lines, and antibodies. All of these are u:aseful in assays for identification of KCNQ2/KCNQ3 protein modulators.

One can administer KCNQ2 and/or KCNQ3 protein modulators to variouus mammalian species, such as monkeys, dogs, cats, mice, rats, humans, etc. By knnown

30 methods, persons skilled in the pharmaceutical art can incorporate KCNQ2/KCNꞥQ3 protein modulators in a conventional systemic dosage form, such as a tablet, capssule,

elixir or injectable formulation. The above dosage forms will also include any

necessary physiologically acceptable carrier material, excipient, lubricant, buffer,r,

antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid or soodium

bisulfite) or the like.

5   **Process of preparation**

In general

This specification describes the cloning and functional expression of fifull-

length human cDNA clones of KCNQ2 (KvLR1) and KCNQ3 (KvLR2), preferahbly

the human KCNQ2 nucleic acid sequence (Figure 2) as shown in SEQ ID NO:3, \ the

10   human KCNQ2 amino acid sequence (Figure 2) as shown in SEQ ID NO:4, the

human KCNQ3 nucleic acid sequence (Figure 23) as shown in SEQ ID NO:17, aand

the human KCNQ3 amino acid sequence (Figure 23) as shown in SEQ ID NO:188.

Also disclosed is a full-length murine cDNA clone of KCNQ2 (murine KvLR1;

Figure 10), preferably the murine KCNQ2 nucleic acid sequence as shown in SECQ ID

15   NO:5, and the murine KCNQ2 amino acid sequence as shown in SEQ ID NO:6.

Additionally, the present invention covers a rat KCNQ2 sequence (Figure 16 andd

Figure 17), preferably the rat KCNQ2 nucleic acid sequence as shown in SEQ ID)

NO:7, and the rat KCNQ2 amino acid sequence as shown in SEQ ID NO:8. The ;

gating kinetics and macroscopic current properties of human, murine and rat KCNNQ2

20   and KCNQ3 currents are similar to those of KCNQ1. However, KCNQ2 and KCCNQ3

are specifically localized within the nervous system and have different

pharmacological properties.

DNA clones comprising nucleotide sequences encoding the following ;

KCNQ2 and KCNQ3 proteins of the present invention were deposited with the

25   American Type Culture Collection ("ATCC") (10801 University Blvd., Manassaas,

VA 20110-2209) on _____, 1998: human KCNQ2, ATCC Accession

Number _____; human KCNQ3, ATCC Accession Number _____; and munrine

KCNQ2, ATCC Accession Number _____. The deposit(s) referred to herein wwill

be maintained under the terms of the Budapest Treaty on the International

30   Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure.e.

These deposits are provided merely as convenience to those of skill in the art andd are

not an admission that a deposit is required under 35 U.S.C. 112. The sequence oof the

polynucleotides contained in the deposited materials, as well as the amino acid

sequence of the of the polypeptides encoded thereby, are incorporated herein by '

reference and are controlling in the event of any conflict with any description of f

5   sequences herein. A license may be required to make, use or sell the deposited

materials, and no such license is hereby granted.

Nucleic acids

With the human KCNQ2, human KCNQ3, murine KCNQ2, and rat

KCNQ2 gene sequences in hand, one skilled in the art can obtain KCNQ nucleicc acids

10   of this invention by known methods. Such methods include: (1) Southern and

Northern blotting; (2) Western immunoblotting; (3) chemical synthesis; (4) syntlthesis

by polymerase chain reaction (PCR) from primers; (5) expression cloning; and ((6)

subtractive cDNA cloning.

Persons skilled in the art can also modify the nucleic acids coding for r the

15   KCNQ proteins of the present invention to prepare useful mutations. For exampple,

one may modify the sequence to provide additional restriction endonuclease

recognition sites in the nucleic acid. Such mutations may be silent or may changge the

amino acid encoded by the mutated codon. One can prepare these modified nucbleic

acids, for example, by mutating the nucleic acid coding for KCNQ2 to result in

20   deletion, substitution, insertion, inversion or addition of one or more amino acidds in

the encoded polypeptide. For methods of site-directed mutagenesis, see Taylor, , J. W.

et al. (1985), Nucl. Acids Res. 13, 8749-64 and Kunkel, J. A. (1985), Proc. Natl.l.

Acad. Sci. USA 82: 482-92. In addition, kits for site-directed mutagenesis are

available from commercial vendors (e.g., BioRad Laboratories, Richmond, CA; ;

25   Amersham Corp., Arlington Heights, IL). For disruption, deletion and truncatioon

methods, see Sayers, J. R. et al. (1988), Nucl. Acids Res. 16: 791-800.

This invention also comprises modified nucleic acids, including (1)

alternative splice exon variants; (2) allelic variants; and (3) chimeric channels inn

which the fusion construct comprises a KCNQ modulatory site. Such modified

30   nucleic acids can be obtained by persons of ordinary skill in the art when armed l with

the present disclosure.

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Expression vectors

This invention further concerns expression vectors comprising a nucleeotide sequence encoding a KCNQ protein of the present invention. Preferably, the expression vectors comprise all or a portion of the nucleic acid sequence as showwn in

5   SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:17.'.

Expression vectors are usually plasmids, but the invention includes otlther vector forms that serve equivalent functions and become known in the art subsequently hereto. A person skilled in the art might also stably integrate a seqquence encoding a KCNQ protein into the chromosome of an appropriate host cell.

10   Expression vectors typically contain regulatory elements capable of affecting expression of a KCNQ protein. These regulatory elements can be heterologous or native KCNQ elements. Typically, a vector contains an origin oof replication, a promoter, and a transcription termination sequence. The vector maay also include other regulatory sequences, including mRNA stability sequences, wḥhich

15   provide for stability of the expression product; secretory leader sequences, whichh provide for secretion of the expression product; environmental feedback sequencces, which allow expression of the structural gene to be modulated (e.g., by the preseence or absence of nutrients or other inducers in the growth medium); marking sequencees, which are capable of providing phenotypic selection in transformed host cells;

20   restriction sites, which provide sites for cleavage by restriction endonucleases; annd sequences which allow expression in various types of hosts, including prokaryotites, yeasts, fungi, plants and higher eukaryotes.

An expression vector of this invention is at least capable of directing tlthe replication, and preferably the expression, of the nucleic acids and protein of thiss

25   invention. Suitable origins of replication include, for example, the Col E1, the SSV4O viral and the M13 origins of replication. Suitable promoters include, for examplvle, the cytomegalovirus promoter, the lacZ promoter, the gal10 promoter and the Autoggrapha californica multiple nuclear polyhedrosis virus (AcMNPV) polyhedral promoterr. Suitable termination sequences include, for example, the bovine growth hormonae,

30   SV40, lacZ and AcMNPV polyhedral polyadenylation signals. Examples of

15

selectable markers include neomycin, ampicillin, and hygromycin resistance and the like.

Persons skilled in the art may insert DNA encoding a KCNQ protein of the present invention into several commercially available vectors. Examples include

5   vectors compatible with mammalian cells, such as pcDNA3 or pCEP4; baculovirus vectors such as pBlueBac; prokaryotic vectors such as pcDNA2; and yeast vectors such as pYes2. For vector modification techniques, see Sambrook et al. (1989), . Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

10   Host cells

This invention additionally concerns host cells containing an expression vector that comprises a sequence encoding a KCNQ protein, preferably the KCNQ2 and/or KCNQ3 proteins of the present invention. The host cells preferably contain an expression vector which comprises all or part of the DNA sequence having the

15   nucleotide sequence substantially as shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:17, particularly the coding regions thereof. Suitable host cells include both prokaryotic cells (e.g., E. coli strains HB101, DH5a, XL1 Blue, Y1090 and JM101) and eukaryotic cells (e.g., Spodoptera frugiperda insect cells, CHO cells, COS-7 cells, HEK 293 cells, human skin fibroblasts, and S.

20   cerevisiae cells).

Persons skilled in the art may introduce expression vectors into host cells by various methods known in the art. Exemplary methods are transfection by calcium phosphate precipitation, electroporation, liposomal fusion, nuclear injection, and viral or phage infection. One may then culture the host cell under conditions permitting

25   expression of large amounts of KCNQ protein.

One may identify such modified host cells by any of six general approaches:

(a) DNA-DNA hybridization with probes complementary to the sequence encoding KCNQ protein (Southern blotting).

30   (b) detection of marker gene functions, such as thymidine kinase activity, resistance to antibiotics, and the like. A marker gene can be placed in the same

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plasmid as the KCNQ sequence under the regulation of the same or a different promoter.

(c) detection of mRNA transcripts by hybridization assays (e.g., Norththern blotting or a nuclease protection assay using a probe complementary to the RNAA sequence).

(d) immunodetection of gene expression (e.g., by Western blotting wirith antibody to KCNQ protein).

(e) detection of potassium channel activity, such as by patch-clamp analysis, radioisotope (e.g., ^{86}Rb) efflux, or membrane potential-sensitive reagernts (e.g., Dibac from Molecular Probes International).

(f) PCR with primers homologous to expression vector sequences or sequences encoding KCNQ protein. The PCR produces a DNA fragment of preedicted length, indicating incorporation of the expression system in the host cell.

Persons skilled in the art may determine DNA sequences by various kknown methods. See, for example, the dideoxy chain termination method in Sanger et al. (1977), Proc. Natl. Acad. Sci. USA 74: 5463-7 and the Maxam-Gilbert method i in Maxam-Gilbert (1977), Proc. Natl. Acad. Sci. USA 74: 560-4.

One may use the host cells of this invention in a variety of ways that aare now apparent. One may use the cells to screen for compounds that bind to or otherwise modulate or regulate the function of KCNQ protein, which would be t useful for modulation, for example activation, of KCNQ2 and/or KCNQ3 protein activvity; to study signal transduction mechanisms and protein-protein interactions; and to pnrepare KCNQ protein for the uses described below.

Not all expression vectors and DNA regulatory sequences will functioon equally well to express the DNA sequences of this invention. Neither will all hoost cells function equally well with the same expression system. However, one of ordinary skill in the art may make a selection among expression vectors, DNA regulatory sequences, and host cells using the guidance provided herein without t undue experimentation and without departing from the scope of the invention.

Polypeptides

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This invention further concerns polypeptides comprising all or a portition of the amino acid sequences of a KCNQ2 and/or KCNQ3 protein. The inventors pprefer polypeptides comprising all or a portion of the amino acid sequences shown as i in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:188.

5   Where a portion of the KCNQ2 and/or KCNQ3 protein is used, preferably the pportion exhibits K^+ channel activity or can be modulated to exhibit K^+ channel activityy. For example, and within the scope of the invention, are polypeptides that comprise ï all or a portion of KCNQ2 and/or KCNQ3 that may contain one or more mutations so tlthat the protein(s) fails to exhibit K^+ channel activity, but that can be used to screen for r

10   compounds that will activate the protein or portion thereof.

Persons having ordinary skill in the art may prepare these polypeptiddes by methods known in the art. For example, one may use chemical synthesis, such ï as the solid phase procedure described by Houghton et al. (1985), Proc. Natl. Acad. Scci. 82: 5131-5. Another method is in vitro translation of mRNA. One may also produace the

15   polypeptides in the above-described host cells, which is the preferred method. FFor example, one may synthesize DNA comprising all or a portion of SEQ ID NO:11, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:17 by PCR as described above, insert tlthe synthesized DNA into an expression vector, transform a host cell with the expreession vector, and culture the host cell to produce the desired polypeptides.

20   Persons skilled in the art can isolate and purify such polypeptides by ï any one of several known techniques; for example, ion exchange chromatography, ggel filtration chromatography and affinity chromatography. Such techniques may reequire modification of the protein. For example, one may add a histidine tag to the prootein to enable purification on a nickel column.

25   Persons skilled in the art can use the polypeptides of the invention in ι a wide variety of ways. For example, one may use them to generate polyclonal or monαoclonal antibodies. One may then use such antibodies for immunodetection (e.g., radioimmunoassay, enzyme immunoassay, or immunocytochemistry), immunopurification (e.g., affinity chromatography) of polypeptides from variouus

30   sources, or immunotherapy (i.e., for potassium channel inhibition or activation).).

Persons skilled in the art may make modified KCNQ polypeptides by known techniques. Such modifications may cause higher or lower activity, permnit higher levels of protein production, or simplify purification of the protein. Such modifications may help identify specific KCNQ2 and/or KCNQ3 amino acids

5   involved in binding, which in turn may help rational drug design of KCNQ2/KCINQ3 modulators. One can make amino acid substitutions based on similarity in polaririty, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include asparticc acid and glutamic acid; positively charged amino acids include lysine and arginine; anmino

10   acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine, glycine, , alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. All suuch modified polypeptides are included within the scope of the invention.

The inventors contemplate a number of other variations of the above-

15   described polypeptides. Such variations include salts and esters of the polypeptiddes, as well as precursors of the aforementioned polypeptides (e.g., having N-terminaal substituents such as methionine, N-formylmethionine and leader sequences). Thae invention includes all such variations.

<u>Method for detecting nucleic acids</u>

20   The present invention further concerns a method for detecting nucleicc acids encoding KCNQ protein. In this method, a person of ordinary skill in the art (a) contacts nucleic acids of unknown sequence with a nucleic acid having a sequenace complementary to a known coding sequence (e.g., a sequence of at least about 100 nucleotides from, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NCO:7,

25   or SEQ ID NO:17, particularly the coding regions thereof), wherein the latter nuacleic acid has a detectable marker; and (b) determines the presence of marker bound too any of the nucleic acids of unknown sequence. The presence of bound marker indicaates the presence of the desired nucleic acids. One can apply this method to detect KCCNQ nucleic acids from other tissues (which may have different regulatory elements) aand

30   nucleic acids from other species (e.g., monkey).

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Persons of ordinary skill in the art generally know how to obtain nucleic acids to be analyzed in this method. For genomic DNA, one can rapidly freeze titissue, crush the tissue into readily digestible pieces, and incubate the crushed tissue in proteinase K and SDS to degrade most cellular proteins. One can then deproteinnize

5   the genomic DNA by successive phenol/chloroform/isoamyl alcohol extractions,:, recover DNA by ethanol precipitation, dry it and resuspend it in buffer. For RN/A, one can lyse cultured cells in 4M guanidinium solution, draw the lysate through ε a 20-gauge needle, pellet the RNA through a cesium chloride step gradient, and remove the supernatant. The pellet should contain purified RNA.

10   The detectable marker may be a radioactive ion linked to one of the nucleotides of the complementary nucleic acid. Common radioactive labels are ^{332}P and ^{35}S, although one may also use other labels such as biotin. Persons skilled iiin the art are aware of various methods to attach the labels to the complementary nucleiːic acid (e.g., the random primer method for attachment of ^{32}P or ^{35}S).

15   Persons of ordinary skill in the art generally know how to carry out suoch a method of detecting nucleic acids. For example, one may perform a Southern or r northern blot using a radiolabeled KCNQ complementary oligonucleotide probe.ː. One can then detect hybridization by autoradiography. Depending on the marker, onee may also use other detection methods (e.g., spectrophotometry).

20   Methods for detecting KCNQ2/KCNQ3 protein modulators

This invention further concerns methods for detecting modulators of thhe KCNQ2 and/or KCNQ3 proteins of the present invention. A screen for KCNQ protein modulators entails detecting binding of molecules (e.g., polypeptides, natntural products, synthetic compounds) in cells expressing KCNQ protein.

25   Cloning and sequencing of KCNQ protein enables construction of cells uaseful in screening for natural products and synthetic compounds that bind to and/or modulate KCNQ protein activity. A process for detecting KCNQ protein moduldators requires transforming a suitable vector into compatible host cells as described previously herein. One treats such transformed cells with test substances (e.g.,

20

synthetic compounds or natural products), and then measures activity in the pressence and absence of the test substance.

Gene Therapy

Persons skilled in the art can also use sense and antisense nucleic acidd

5   molecules as therapeutic agents for KCNQ-related indications. One may constrmuct vectors that direct the synthesis of the desired DNA or RNA or formulate the nuacleic acid as described in the art.

Several references describe the usefulness of antisense molecule. See Toulme and Helene (1988), Gene 72: 51-8; Inouye (1988), Gene, 72: 25-34; Uhlilmann

10   and Peyman (1990), Chemical Reviews 90: 543-584; Biotechnology Newswatchh (January 15, 1996), p. 4; Robertson, Nature Biotechnology 15: 209 (1997); Gibbbons and Dzau (1996), Science 272: 689-93. One can design them based on genomic l DNA and/or cDNA, 5' and 3' flanking control regions, other flanking sequences, intronn sequences, and nonclassic Watson and Crick base pairing sequences used in formmation

15   of triplex DNA. Such antisense molecules include antisense oligodeoxyribonucleotides, oligoribonucleotides, oligonucleotide analogues, andd the like, and may comprise at least about 15 to 25 bases.

Antisense molecules may bind noncovalently or covalently to the KCΓNQ DNA or RNA. Such binding could, for example, cleave or facilitate cleavage of f

20   KCNQ DNA or RNA, increase degradation of nuclear or cytoplasmic mRNA, oor inhibit transcription, translation, binding of transactivating factors, or pre-mRNAA splicing or processing. Antisense molecules may also contain additional functionalities that increase stability, transport into and out of cells, binding affirinity, cleavage of the target molecule, and the like. All of these effects would decreasse

25   expression of KCNQ protein and thus make the antisense molecules useful as KCCNQ protein modulators.

Detailed Description of Preferred Embodiments

Human KCNQ2 and KCNQ3

Genetic properties

KCNQ1-Related (KCNQ2/KCNQ3) expressed sequence tags (ESTs) weere

5   discovered by a GCG BLAST search of the GenBank database with KCNQ1

sequence. Primers, derived from the consensus sequences of EST clones, were uused to

amplify human brain-derived cDNA and 877 bp and 325 bp fragments were isoblated

for KCNQ2 and KCNQ3, respectively. (Figure 1, probe I). To obtain full-lengtth

cDNA sequences of both genes, we employed 5'RACE PCR, screening of cDNA

10   libraries, and Gene Trapper techniques. The composite full-length cDNAs of KCCNQ2

(SEQ ID NO:3) and KCNQ3 (SEQ ID NO:17) contain an open reading frame (CORF)

encoding an 871 (SEQ ID NO:4) and 854 (SEQ ID NO:18) amino acid polypepbtide,

respectively (Figure 2 and Figure 23). DNA sequence analysis and conceptual

translation of both cDNAs reveals that they encode proteins with the structural

15   features of a voltage-gated potassium channel and are most closely related to KCCNQ1.

Sanguinetti et al. (1996), Nature 384: 80-83; Yang et al. (1997), Proc. Natl. Acaad. Sci.

USA 94:4017-2. KCNQ2 exhibits a high degree of sequence similarity with KCCNQ3

($\approx$70%), indicating that they belong to the same subfamily. Both proteins have aa

longer C-terminal domain (~200 amino acids) than KCNQ1. The initiation coddon for

20   KCNQ2 is flanked by a consensus ribosome binding site (i.e., Kozak) ACCATCGG

(Figure 2).

At the amino acid level, sequence analysis reveals that KCNQ2/KvLR1

contains the GYG (i.e., Gly-Tyr-Gly) potassium channel pore "signature sequennce"

and, therefore, is likely to encode a potassium-selective channel. A comparison i of

25   KCNQ2 and KCNQ1 (KvLQT1) reveals that the amino acid sequence identity iris

approximately 60% in the transmembrane and pore regions (Figure 3). KCNQ33

exhibits about the same degree of identity (about 56%) with KCNQ1 as KCNQ22 in

the transmembrane and pore regions (Figure 4). The identity in the amino-termininal

and carboxy-terminal domain is much less compared to the central conserved reegions

30   (Figure 3). Such findings suggest that KCNQ2/KvLR1 and KCNQ3/KvLR2 aree

additional members of the KCNQ1/KvLQT1 family of ion channels.

KCNQ2- and KCNQ3-specific transcripts are detectable only in humaan
brain (Figure 5). This expression pattern is distinct from KCNQ1/KvLQT1, whiiich is
expressed strongly in human heart and pancreas as revealed by Northern blot anaalysis.
Sanguinetti et al. (1996) Nature 384: 80-83; Yang et al. (1997), Proc. Natl. Acadd. Sci.

5   USA 94:4017-2. Expression of human KCNQ2/KvLR1 is high in the hippocamppus,
caudate nucleus, and amygdala; moderate in the thalamus; and weak in the
subthalamic nucleus, substantia nigra and corpus callosum (Figure 5). A separatite
Northern blot demonstrates that expression of human KCNQ2/KvLR1 is high in a the
cerebral cortex; is moderate in the putamen, temporal lobe, frontal lobe, occipitahl pole

10   and cerebellum; and is low in the medulla and spinal cord (Figure 5). KCNQ3
exhibits a nearly identical expression pattern in the brain (Figure 5). In order to ·
characterize further the cell types that express KCNQ2/KvLR1, a murine-specifiúc
KCNQ2/KvLR1 cDNA fragment was isolated and used as an in situ hybridizatioon
probe. The result (Figure 9) shows that KCNQ2/KvLR1 is expressed in the mouuse

15   hippocampus and dentate gyrus, areas that are important in learning and memoryy.

Electrophysiological properties

The full-length human KCNQ2 and KCNQ3 cDNAs were subcloned i into a
Xenopus expression vector and cRNA was generated by in vitro transcription. TThe
properties of the channels encoded by human KCNQ2 and KCNQ3 were investijgated

20   by expressing the transcribed cRNA in Xenopus oocytes. Figure 6 compares cunrrents
recorded from oocytes that were injected 5 days earlier with either water (Figuree 6A)
or 14 ng of human KCNQ2/KvLR1 cRNA (Figure 6B). Oocytes injected with hhuman
KCNQ2/KvLR1 cRNA exhibited outward currents that activated at potentials poositive
to -60 mV and had a maximal amplitude of 1 µA at +40 mV. Similar currents wvere

25   never observed in water-injected control oocytes and small leak or endogenous
currents recorded in control oocytes never exceeded 0.15 mA at +40 mV. The hnuman
KCNQ2/KvLR1 currents exhibited a rapidly activating delayed rectifier current :
phenotype very similar to hKCNQ1/KvLQT1 current. Barhanin et al. (1996) Naature
384: 78-80; Sanguinetti et al. (1996), Nature 384: 80-83; ; Yang et al. (1997) Prooc.

30   Natl. Acad. Sci. USA 94:4017-2. KCNQ2/KvLR1 current rectified weakly at poositive
voltages.

Although the macroscopic KCNQ2/KvLR1 and KCNQ1/KvLQT1 cunrrents are similar, KCNQ2/KvLR1 tail currents lack the "hook" observed with KCNQ1/KvLQT1 tail current. Figure 6C shows the peak current-voltage (IV) relationship for oocytes expressing KCNQ2/KvLR1 (n = 12). The K$^+$ selectivity,y of
5   the expressed current was examined by investigation of tail current reversal poteentials in bath solutions containing 2, 10, 40 and 98 mM K$^+$. Reversal potentials closelyy followed the Nemst potential for K$^+$ revealing a K$^+$-selective channel (n = 6; Figgure 6D). The reversal potential for KCNQ2/KvLR1 current shifted by 52 mV per 100-fold change in external K$^+$. The dashed line has a slope predicted from the Nemst eqquation
10   for a perfectly selective K$^+$ channel.

A family of currents elicited by depolarizing voltage steps in an oocyte injected with KCNQ3 cRNA are shown in Figure 18A. The currents activate at : potentials positive to -70 mV and rectify inwardly at potentials greater than 0 mVV, as is obvious from the IV relationship (Figure 18B). The KCNQ3 reversal potentiaal
15   shifted 49 mV per 10-fold change in external K$^+$ (Figure 18C). Thus, although still predominantly selective for K$^+$, KCNQ3 is slightly less K$^+$-selective than KCNQQ2.

Co-expression of KCNE1 (KCNE1 is also known as "minK" or "Isk")') with KCNQ1/KvLQT1 dramatically alters the amplitude and gating kinetics of KCNQ1/KvLQT1 current. Barhanin et al. (1996) Nature 384: 78-80; Sanguinetttti et
20   al. (1996), Nature 384: 80-83; ; Yang et al. (1997) Proc. Natl. Acad. Sci. USA 94:4017-2. MinK is a polypeptide thought to encode or regulate a K$^+$ channel. Folander et al. (1990) Proc. Natl. Acad. Sci. USA 87: 2975-2979; Varnum et al. . (1993) Proc. Natl. Acad. Sci. USA 90: 11528-11532; Ben-Efraim et al. (1996) J.I. Biol. Chem. 271: 8768-8771. These studies suggest that minK and KCNQ1/KvLQT11 co-
25   assemble to form the K$^+$ channel underlying the slow delayed rectifier current inn heart. A similar association between minK and KCNQ2/KvLR1 was tested. Coexpresssion of KCNE1 with KCNQ2/KvLR1 had little effect on the KCNQ2/KvLR1 currentıt in oocytes, and separate currents carried by KCNQ1/KvLQT1 and KCNQ2/KvLR11 channels could be delineated in oocytes co-injected with minK and KCNQ2/KvIrLR1
30   using selective inhibitors for each of the channels. Thus, KCNQ2/KvLR1 interaacts

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differently with KCNE1 than does KCNQ1/KvLQT1. Different KCNQ memberrs may functionally interact with proteins structurally similar to KCNE1.

Pharmacological properties

Inhibitors of various potassium channels present in brain and other tisssues
were used to investigate the pharmacology of KCNQ2/KvLR1. The effects of 0.2 mM of 4-aminopyridine (4-AP), 10 μM E-4031, 10 μM clofilium, 0.1 mM of charybdotoxin, and 1 mM tetraethylammonium (TEA) on KCNQ2/KvLR1 currernts recorded from a single oocyte are shown in Figure 7. Each of these compounds vwas also tested alone in individual oocytes and the effects of each agent were no diffeerent.

Charybdotoxin is a scorpion venom protein that inhibits a variety of Ca^{2+}-activated and voltage-dependent K^+ channels. Miller et al. (1985), Nature 313: 3316-8; Sugg et al. (1990), J. Biol. Chem. 265: 18745-8. Charybdotoxin did not inhibit tlthe KCNQ2/KvLR1 current at the concentration tested. This toxin also had no effectt on KCNQ1/KvLQT1.

E-4031 (10 mM) is a selective inhibitor of I_{Kr}. Sanguinetti et al. (19900) J. Gen. Physiol. 96: 195-215). 4-AP (0.2 mM) is an inhibitor of Shaker-type K^+ channels. Deal et al. (1996) Physiol. Rev. 76: 49-67. Neither E-4031 nor 4-AP produced significant effects on KCNQ2/KvLR1 current. Similarly, both reagentss do not inhibit KCNQ1/KvLQT1 currents. Yang et al. (1997), Proc. Natl. Acad. Sci., USA 94:4017-21.

TEA is a weak inhibitor of KCNQ1/KvLQT1 whereas clofilium is a sttrong inhibitor of KCNQ1/KvLQT1. Yang et al. (1997), Proc. Natl. Acad. Sci. USA 944:. Clofilium also inhibits cardiac I_{Kr} and I_{Ks}. Arena et al. (1988), Molecular Pharmacology 34: 60-66; Colatsky et al. (1990), Circulation 82: 2235-42. For KCNQ2/KvLR1, in contrast, clofilium had little effect whereas TEA inhibited thae current by over 85% at a concentration of 1 mM.

The pharmacology of KCNQ3 was significantly different than that of KCNQ2 (Figure 18D). Clofilium (10 μM) reduced KCNQ3 current by 30% fromm control but had little effect on KCNQ2. TEA, which strongly inhibited KCNQ2 ɛ at 1 mM, produced little inhibition of KCNQ3 at 5mM. CTX (100 nM), 4-AP (2 mMM) and E-4031 (10 μM) also had no effect on KCNQ3 current.

As can be seen from these results, the pharmacological properties of KCNQ3/KvLR2, KCNQ2/KvLR1 and KCNQ1/KvLQT1 are quite different.

KCNQ2 and KCNQ3 functionally interact

The overlapping expression pattern of KCNQ2 and KCNQ3 in different t brain regions (Figure 5), prompted us to test for functional interaction between the twco channels. Families of currents elicited by depolarizing voltage steps in oocytes injected with KCNQ2 and KCNQ3 alone and together are shown in Figure 19A . through Figure 19C. Current amplitudes recorded from oocytes co-expressing tt:he two channels were 15-fold greater than in oocytes injected with each of the channels ; individually. Peak current amplitudes at +30 mV for KCNQ2, KCNQ3 and KCNQ2+KCNQ3 were 0.98±0.09 (n=6), 0.98±0.06 (n=5) and 14.2±0.62 μM (n=6), respectively. Quantitatively similar results were obtained in 3 separate batches oof oocytes. The IV relationship shows that KCNQ2+KCNQ3 currents activated at : potentials positive to -60 mV and did not rectify, unlike KCNQ2 and particularlyy KCNQ3, at positive voltages (Figure 19D). The reversal potential of tail currentuts shifted by 57 mV per 10-fold change in external K^+ indicating that KCNQ2+KC:NQ3 is nearly perfectly selective for K^+ (Figure 19E). KCNQ2+KCNQ3 current is wveakly sensitive to inhibition by 5 mM TEA and 10 μM clofilium but not to 100 nM CTTX or 2 mM 4-AP (Figure 19F). E-4031 (10 μM) also did not inhibit KCNQ2+KCNQ3 current (not shown). These results suggest strongly that KCNQ2+KCNQ3 interaact to form a channel with properties distinct from either KCNQ2 or KCNQ3 channelss alone.

KCNE1 interacts with KCNQ2+KCNQ3 channels

The β subunit KCNE1 dramatically alters the amplitude and gating kinetitics of the KCNQ1 channel. Barhanin et al. (1996) Nature 384: 78-80; Sanguinetti et al.l. (1996), Nature 384: 80-83; Yang et al. (1997) Proc. Natl. Acad. Sci. USA 94:40)17-2; Romey et al. (1997) J. Biol. Chem. 272:16713-16716. Because KCNQ2 and KCCNQ3 are members of the same K^+ channel subfamily, we tested for an interaction betvween KCNE1 and KCNQ2+KCNQ3 channels. Figure 20 shows currents elicited by 1 l sec depolarizing voltage steps in oocytes expressing KCNE1 alone (Figure 20A),

KCNQ2+KCNQ3 (Figure 20B), and KCNQ2+KCNQ3+KCNE1 (Figure 20C).

KCNE1 significantly attenuated KCNQ2+KCNQ3 current amplitude and slowedd

gating kinetics. Peak current amplitude at +30 mV was reduced by 62±6.0 % (n=6) in

oocytes co-expressing KCNE1. Activating currents were fitted to a bi-exponentitial

5   function to determine fast and slow time constants of activation. Fast and slow titime

constants for activation of KCNQ2+KCNQ3 current at +10 mV were 50.1±3.4 (n=6)

and 239.3±17.5 ms (n=6), respectively; these were shifted to 124.7±8.8 (n=5) annd

680.7±71.4 ms (n=6) when KNCE1 was injected together with KCNQ2+KCNQ33.

Similar results were obtained in more than 15 oocytes from each group in this annd two

10   additional batches of oocytes. KCNE1 currents appear absent because of the durration

(1 sec) of the voltage steps used and the scale at which the currents are shown.

However, as shown clearly in the inset in Figure 20A, 5 sec voltage steps elicitedd

typical KCNE1 currents in the same oocyte. The effect of KCNE1 on gating kinnetics

is similar for KCNQ1 and KCNQ2+KCNQ3 channels. In contrast, KCNE1 augrments

15   KCNQ1 current but inhibits KCNQ2+KCNQ3.

The results explain why mutations in either of two unlinked K^+-channel

encoding genes yield the same phenotype. BFNC-associated mutations in eitherr

KCNQ2 or KCNQ3 could cause a profound reduction in KCNQ2+KCNQ3 curreent

amplitude. One study has shown that a BFNC-causing mutation resulting in an

20   nonfunctional, truncated KCNQ2 protein, failed to produce a dominant-negative

inhibition of wild-type KCNQ2 channels expressed in oocytes. Biervert et al. (19998),

Science 279:403-406. The present invention, demonstrating a synergistic interaocction

between KCNQ2 and KCNQ3, may provide a likely explanation for this finding.;. That

is, mutations in KCNQ2 may only produce dominant-negative effects when co-

25   expressed with wild-type KCNQ3 channels, and vice versa.

Molecular genetics

Recent advances in molecular genetics has allowed us to correlate

potassium channels with diseases in the nervous system. Most recently, and as

discussed above, BFNC, a class of idiopathic generalized epilepsy, was recently '

linked to mutations in KCNQ2 and KCNQ3. Biervert et al., supra; Charlier et al., supra; and Singh et al., supra. The identification and expression of human KCNQ2 and human KCNQ3 will allow us to investigate further correlations with BFNC and other potential human disease. The present invention will now permit those skilled in

5   the art to identify modulators, e.g., activators, of KCNQ2 and/or KCNQ3. Modulators of KCNQ2 and/or KCNQ3 may provide opportunity for treatment of disease, such as BFNC. Additionally, because human KCNQ2 and KCNQ3 are expressed highly in areas associated with learning and memory, modulators of KCNQ2 and/or KCNQ3 may also provide opportunity for pharmacological treatment

10   of the memory loss associated with advanced age, Parkinson's disease or Alzheimer's disease.

Murine KvLR1

Starting with a brain expressed sequence tag (EST, public domain database)

15   similar to the KvLQT1 gene, a novel potassium channel gene was cloned from a mouse brain library and functionally expressed. Figure 10A through Figure 10D shows the murine KCNQ2/KvLR1 gene (SEQ ID NO:5) encoding for a protein of 722 amino acids (SEQ ID NO:6) and a calculated molecular weight of 80.4 kDa. Hydropathy analysis (Figure 10E) illustrates the computer-generated topology of

20   KvLR1 to have 6 membrane spanning domains and a pore domain typical of voltage-gated potassium channels.

The amino acid alignment of the murine KCNQ2/KvLR1 channel with the murine KCNQ1/KvLQT1 channel is shown in Figure 11. Overall, there is 40% identity between the two channels with 62.5% identity within the spanning and pore

25   domains. Phylogenetic analysis suggests that the murine KCNQ2/KvLR1 gene to be a member of the KCNQ1/KvLQT1 gene family and to be distantly related to the HERG gene and other voltage-gated family members. Signature amino acid sequences characteristic of voltage-gated potassium channels are present within murine KCNQ2/KvLR1; a repeating arginine pattern is seen within the S4 spanning

30   domain known as the voltage sensor, and a GYG sequence within the pore region. Further analysis of several 3' RACE clones indicate diversity past the S6 membrane

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spanning domain. To date, two alternative splice exons, A and B, have been identified, the amino acid sequences of which are shown in Figure 12.

To determine tissue distribution of murine KCNQ2/KvLR1, a northern bblot was performed with a probe from the murine KCNQ2/KvLR1 channel that did nnot

5   contain the pore or voltage sensor regions. This sequence of the gene avoids posssible cross-reactivity with other channels. The results, shown in Figure 13, indicate a a highly abundant 8.2 kb message found only in the brain and not observed in peripheral tissues. Although not absolute, longer exposures of the northern blot t did not indicate the presence of the message in the peripheral tissues indicated in Figgure 5.

10   To obtain higher resolution of message localization within the brain, in ssitu hybridization was performed. Positive hybridization signal with an antisense riboprobe specific for a nonconserved region of the KCNQ2/KvLR1 gene is obsserved with a broad distribution throughout much of the rat brain. The mouse probe waas 99% identical to the rat sequence. Robust signal, however, is observed with a mnore

15   limited distribution in the following regions: piriform cortex, supraoptic nucleuas, amygdala, hippocampus, including the CA1, 2, and 3 regions and the dentate gyyrus, MO5 (motor nucleus of the brain stem trigeminal), facial nucleus, hypoglossal nucleus, inferior olivary nuclei, deep cerebellar nuclei, gigantocellular nuclei, latteral and medial vestibular nuclei, motor neurons of the spinal cord, and sensory neurrons of

20   the dorsal root ganglion. Moderate levels of hybridization signal are also observved in the cortex, septum, striatum, hypothalamus, thalamus, medial habenula, substantitia nigra compacta, mammillary nuclei, lateral and medial geniculate, interfasicular r nucleus, purkinje and granule cells of the cerebellum, parabrachial nuclei, dorsalil and ventral cochlear nuclei, and other brain stem nuclei. A composite view of three

25   regions is shown in Figure 14.

To test for functional expression, cRNA was prepared from the murine KCNQ2/KvLR1 gene and injected into Xenopus oocytes. In a two-electrode vobltage clamp, a family of outward currents were generated in murine KCNQ2/KvLR1 cRNA-injected oocytes (n>20). After a minimum of 48 hours, currents qualitatitively

30   and quantitatively different than native currents generated with identical protocools in water injected or uninjected control cells (representing Ca^{2+} -activated chloride

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currents and other native currents) (Figure 15). The murine KCNQ2/KvLR1-mediated currents were blocked by 1 mM TEA. Similar currents were obtained1 from CHO cells stably expressing murine KCNQ2 and recorded using patch-clamp techniques. Single channel conductances were estimated to be 24-30 pS in

5   symmetrical 140 mM potassium. (Figure 22).

To determine if murine KCNQ2/KvLR1 has similar pharmacology to I_{Ks} and I_{Kr} currents in cardiac myocytes, clofilium was tested on oocytes expressingg murine KCNQ2. At 20 μM, clofilium was shown to partially block the murine KCNQ2-mediated currents. Other specific K^+ channel blocking toxins, includinng

10   iberiotoxin, α-dendrotoxin and charybdotoxin, had no significant effect on muririne KCNQ2-mediated currents.

Materials and Methods

Human KCNQ2

15   #### Molecular cloning and expression of human KCNQ2 (human KvLR1) and humaan KCNQ3 (human KvLR2)

5' RACE PCR was performed by amplifying human brain or fetal braain cDNA libraries or Marathon-Ready cDNAs (Clontech) using primers derived frrom the KvLQT1-related EST sequences (EST# yn72g11, yo31c08, ys93a07 (sequencess can

20   be found in Genbank database)) (Figure 1). PCR products were gel-purified, subcloned and sequenced. Random-primed ^{32}P-labelled DNA probes containinng specific regions of KCNQ2 or KCNQ3 sequence were used for screening of cDNA libraries and Northern blot analysis using standard protocol. For example, KCNNQ2 Probe I (Figure 1) was used for Northern blot analysis; Probe II (Figure 1) was uused

25   for screening human brain cDNA libraries according to standard protocols.

The Gene Trapper experiment was performed using the protocol as described in the Manufacturer's manual (LifeTechnologies). The composite fullll-length human KCNQ2 and human KCNQ3 cDNA clones were obtained by restritriction enzyme digestion and ligation of overlapping cDNA clones. The full-length cDDNAs

30   were subcloned into a Xenopus expression vector, derived from pSP64T plasmidd.

Capped cRNA for microinjection was synthesized using mMESSAGE mMACHIINE
Kit (Ambion).

　　　　　For detection of expression of KCNQ2 as shown in Figures 9A and 9BB,
tissue processing, histological analyses and in situ hybridization analyses were
5　　performed essentially as described in Fagan et al. (1996), J. Neurosci. 16 (19): 63208-
18.

Electrophysiological and pharmacological characterization of KCNQ2 and KCNQQ3

　　　　　Stage V and VI Xenopus laevis oocytes were defolliculated with
10　　collagenase treatment and injected with cRNAs, as described in Yang et al., suprra.
Currents were recorded at room temperature using the two-microelectrode voltagge
clamp (Dagan TEV-200) technique between 3-5 days after injection of KCNQ2 ((15
ng), KCNQ3 (15 ng), or KCNE1 (2 ng) cRNA alone or in combination.
Microelectrodes (0.8 to 1.5 MΩ) were filled with 3 M KCl. Bath solution contaiiined
15　　(in mM): 96 NaCl, 2 KCl, 0.4-1.8 CaCl$_2$, 1-2 MgCl$_2$ and 5 HEPES (pH 7.5). KCCl
was varied in some experiments by equimolar substitution with NaCl.

　　　　　K$^+$ selectivity was assessed by determining the dependence of tail currrrent
reversal potential on the external K$^+$ concentration. Tail currents were elicited aßt
potentials of -110 to +10 mV following a voltage step to +20 mV while the externrnal
20　　K$^+$ concentration was varied between 2, 10, 40, and 98 mM. Current reversal
potential under each condition was determined for each oocyte by measuring thes zero
intercept from a plot of tail current amplitude vs test potential.

　　　　　Axoclamp (Axon Instruments) was used for generating voltage clamp ı
commands and acquiring data and Axograph 3.0 (Axon Instruments) was used fdor
25　　data analysis. All data was sampled at rates at least two times the low pass filterr rate.
Experiments were performed at 22-25 °C. Clofilum was obtained from RBI
Biochemicals and 4-aminopyridine (4-AP), TEA and charybdotoxin were obtainaed
from Sigma Chemical Co. E-4031 was synthesized from information published l by
Esai Research Laboratories.

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Murine KvLR1

Probe preparation and library screening

A unique expressed sequence tag (EST) was identified from the public

database that has similarity to the KvLQT gene. Oligonucleotide primers were

5   synthesized from the EST sequence for PCR experiments. The forward primer (SSEQ.

ID. NO.: 15) was

5'-GAG TAT GAG AAG AGC TCG GA-3'

and reverse primer (SEQ. ID. NO.: 16) was

5'-CAG ATG TGG CAA AGA CGT TGC-3'.

10 Rat brain polyA$^+$ RNA was reverse-transcribed with random hexamers and ampli:ified

by PCR [60 sec 94 °C, 90 sec 55 °C, 120 sec 72 °C, 30 cycles] with the above

primers. A 240 bp DNA fragment of rat KCNQ2/KvLR1 was isolated by gel

electrophoresis and subcloned into pCRII (InVitrogen). The 240 bp DNA fragmeent

was random-prime labeled with ^{32}P-dCTP and used as a probe to screen a mouse

15 brain pcDNA1 plasmid library (Clontech, Palo Alto, CA). Overall, 2×10^5 colonides

were screened using standard filter lift protocols. The filters were hybridized

overnight in 50% formamide, 2X PIPES and 1% SDS at 42 °C and washed 1x in ¡ 1X

SSC then 3x 20 minutes in 0.1x SSC, 0.1% SDS at 53 °C. Filters were exposed

overnight at -70 °C. Only one positive colony was identified and replated until

20 purified. Clone mbr 26.1, designated murine KvLR1, was sequenced on both straands

by dideoxy termination reactions.

Northern Blots

Northern blots were performed with the mouse multiple tissue blot (Clonttech)

according to the manufacturer's instructions. Briefly, the blot was prehybridized 1 at

25 68°C with ExpressHyb solution for 30 minutes. A DNA fragment was isolated fifrom

the murine KvLR1 coding region by the restriction enzyme PvuII, which eliminatted

the pore and voltage sensor consensus sequences, and random-prime labeled withh ^{32}P-

dCTP, denatured at 100°C for 5 minutes, chilled on ice and added to fresh

ExpressHyb before addition to the northern blot. The blot was incubated for 60

30 minutes at 68°C with continuous shaking. The blot was washed 2X at 50°C in 0.¡.1X

SSC and 0.1% SDS. The blot was wrapped in saran wrap and exposed to x-ray fifilm

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overnight at room temperature. The same protocol was used for the actin probe
provided with the blot.

In situ hybridization

Frozen sections cut at intervals of 225 µm through the entire adult rat braain

5   were fixed by immersion (without thawing) into ice cold 10% formaldehyde in FPBS
for 20 minutes and rinsed with PBS. Fixed sections of rat DRG were treated witith
0.5% Triton X-100 in 0.1 M Tris, pH 8.0, and 0.05 M EDTA for 30 minutes andd
rinsed for 3 minutes in 0.1 M Tris, pH 8.0, and 0.05 M EDTA. The tissue was tthen
treated with 0.1 M TEA, pH 8.0, plus 0.25% acetic anhydride for 10 minutes at rroom

10 temperature, rinsed (3 X) in 2X SSC, dehydrated through a series of alcohols,
delipidated in chloroform, and air dried.

Riboprobes were synthesized using the Promega Riboprobe Transcriptioon
System II with 250 µCi ^{35}S-UTP and 250 µCi ^{35}S-CTP in a total reaction volunme of
10 µL. Unlabeled UTP and CTP were added at 25 µM each and ATP and GTP aat 500

15 µM each. The murine KCNQ2/KvLR1 plasmid (nts 552-1125 subcloned into
pBluescript II) was linearized with Sac I and transcribed using T3 RNA polymerrase,
and with BamHI and transcribed using SP6 RNA polymerase to generate anti-seense
and sense probes, respectively. One µg of linearized plasmid was added for eactth
reaction. The riboprobes were purified by phenol:chloroform extraction and twoo

20 ethanol precipitations using ammonium acetate. The dried tissue sections were
hybridized with 1 X 10^{7} cpm/ml riboprobe in hybridization buffer (50% formanmide,
0.3 M NaCl, 10 mM Tris, 1 mM EDTA, 1X Denhardt's solution, 10% dextran suulfate,
500 µg/ml tRNA and 10 mM DTT) overnight at 55°C. The hybridization solutidon
was removed by rinsing 4 times in 4X SSC, 5 minutes for each wash. The sectioons

25 were incubated in 0.02 mg/ml RNase, 0.5 M NaCl, 10 mM Tris, pH 8.0, and 1 mmM
EDTA for 30 minutes at 37°C, then washed in 2X SSC, 1X SSC and 0.5X SSC, , all
containing 1 mM DTT, for 10 minutes per wash at room temperature. The tissuaes
were incubated in 0.1X SSC, 1 mM DTT for 30 minutes at 55°C, then rinsed briaefly
in 0.1X SSC and 1 mM DTT at room temperature, dehydrated, and air dried. Thhe

30 dried sections were exposed to XOMAT film (Kodak, Rochester, NY), then werre

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dipped in NTB2 emulsion (Kodak, Rochester, NY) to determine the cellular localization of each mRNA.

Expression and Recording in Oocytes

The murine KCNQ2/KvLR1 cDNAs were linearized with the restrictionn

5   enzyme NotI and in vitro transcribed using the mMessage mMachine T7 RNA ι polymerase kit according to the manufacturer's instructions (Ambion, Austin, TTX). The cRNAs were solubilized in RNase-free water, and stored at –70 °C at a concentration of 1.0 μg/μl. Frog oocytes were prepared and injected using stanndard techniques (Colman, 1984). In murine KvLR1 expression experiments, each ooocyte

10   was injected with approximately 35-40 nl of the cRNA. Following injection, ooocytes were maintained at 17°C in ND96 medium consisting of (in mM): NaCl, 90; KCCl, 1.0; CaCl$_2$, 1.0; MgCl$_2$, 1.0; HEPES, 5.0; pH 7.5. Horse serum and penicillin/streptomycin, both 5% of final volume, were added as supplements too the incubation medium. Electrophysiological recording commenced 2-6 days folloowing

15   cRNA injection. Prior to the start of an experiment oocytes were placed in a recording chamber and incubated in Modified Barth's Solution (MBS) consistinng of (in mM): NaCl, 88; NaHCO$_3$, 2.4; KCl, 1.0; HEPES, 10; MgSO$_4$, 0.82; Ca(NO$_3$)$_2$, 0.33; CaCl$_2$, 0.41; pH 7.5. Oocytes were impaled with electrodes (1-2 MΩ) annd standard 2-electrode voltage clamp techniques were employed to record whole-ᴄ-cell

20   membrane currents (Stuhmer, 1992; TEC 200, Dagan Instruments). Voltage-cldamp protocols typically consisted of a series of voltage steps 100-500 ms duration, irin +10 mV steps from a holding potential of -60 mV to -90 mV to a maximal potentiall of +40 mV to +50 mV; records were digitized at 5 kHz and stored on a computer usingg pClamp 6.0 software (Axon Instruments), and analyzed using ClampFit or AxobGraph

25   software (Axon Instruments).

Expression and Recording in CHO cells

Patch clamp recordings were obtained from CHO cells that transiently oor stably expressed murine KCNQ2 channels. Electrodes were prepared using a PPC-84 Sachs-Flaming pipette puller (Sutter Instruments) and fire-polished to a final tipp

30   resistance of 3-5 MΩ. Pipettes were filled with a solution that consisted of (in rmM)

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KCI (140), MOPS (20), K₂EGTA (1.0), CaCI₂ (0.89), pH 7.2. The pipette solutition

sometimes contained MgCI₂ (1.0) to aid in seal formation. Cells were grown onn poly-

D-lysine coated coverslips, and pieces of the coverslips containing CHO cells wvere

placed into a chamber on an inverted microscope for recording. Prior to recordiring,

5   and during seal formation, cells were bathed in an external solution consisting obf (in

mM) NaCI (145), KCI (3), CaCI₂ (2.5), MgCI₂ (1.0), HEPES (10), pH 7.4. Elecctrodes

were lowered to the surface of cells under visual inspection; following gigaseal

formation inside-out membrane patches were excised into an internal solution

consisting of (in mM) KCl (140), MOPS (20), K₂EGTA (1.0), CaCI₂ (0.89), pH I 7.2.

10 All recordings were made under symmetrical K^+ conditions. Following patch

excision continuous and step-protocol voltage-clamp recordings were obtained, ¿ and

analyses performed, using an AxoPatch 200B Patch Clamp amplifier and pClammp

software (Axon Instruments). Results are shown in Figure 22.

Although the present invention has been described in some detail by wayy of

15 illustration and example for purposes of clarity and understanding, it will be appparent

that certain changes and modifications may be practiced within the scope of the ¿

appended claims.

35

What is claimed is:

1.   An isolated nucleic acid molecule encoding a polypeptide comprising allll or a portion of a KCNQ protein, wherein said KCNQ protein is selected from the grooup

5   consisting of human KCNQ2 protein, human KCNQ3 protein, murine KCNQ2 protein, and rat KCNQ2 protein.

2.   The nucleic acid molecule of claim 1 wherein said human KCNQ2 proteẹin comprises the amino acid sequence as shown in SEQ ID NO:4.

3.   The nucleic acid molecule of claim 1 wherein said human KCNQ3 proteẹin

10   comprises the amino acid sequence as shown in SEQ ID NO:18.

4.   The nucleic acid molecule of claim 1 wherein said murine KCNQ2 proteẹin comprises the amino acid sequence as shown in SEQ ID NO:6.

5.   The nucleic acid molecule of claim 1 selected from the group consisting (of: (a) all or a portion of a nucleic acid sequence as shown in SEQ ID NO:3; (b) the

15   complement of (a); and (c) variations of (a) due to degeneracy in the genetic codde.

6.   The nucleic acid molecule of claim 1 selected from the group consisting (of: (a) all or a portion of a nucleic acid sequence as shown in SEQ ID NO:17; (b) the complement of (a); and (c) variations of (a) due to degeneracy in the genetic codde.

7.   The nucleic acid molecule of claim 1 selected from the group consisting (of: (a)

20   all or a portion of a nucleic acid sequence as shown in SEQ ID NO:5; (b) the complement of (a); and (c) variations of (a) due to degeneracy in the genetic codde.

8.   A vector comprising the nucleic acid molecule of claim 1.

9.   A vector comprising the nucleic acid molecule of claim 2.

10.   A vector comprising the nucleic acid molecule of claim 3.

25   11.   A vector comprising the nucleic acid molecule of claim 5.

12.   A vector comprising the nucleic acid molecule of claim 6.

13.   A prokaryotic or eukaryotic host cell comprising the vector of claim 8.

14.   A prokaryotic or eukaryotic host cell comprising the vector of claim 9.

15.   A prokaryotic or eukaryotic host cell comprising the vector of claim 10. .

30   16.   A prokaryotic or eukaryotic host cell comprising the vector of claim 11. .

17.   A prokaryotic or eukaryotic host cell comprising the vector of claim 12. .

18.   An isolated KCNQ protein or polypeptide comprising all or a portion of aan amino acid sequence selected from the group consisting of human KCNQ2 as shaown in SEQ ID NO:4, human KCNQ3 as shown in SEQ ID NO:18, murine KCNQ2 aas shown in SEQ ID NO:6, and rat KCNQ2 as shown in SEQ ID NO:8.

5   19.   An isolated nucleic acid molecule selected from the group consisting of: ((a) a nucleotide sequence that exhibits at least 80% sequence identity to the nucleotidee sequence as shown in SEQ ID NO:3; (b) a nucleotide sequence that exhibits at leeast 80% sequence identity to the nucleotide sequence as shown in SEQ ID NO:17; annd (c) a nucleotide sequence that exhibits at least 80% sequence identity to the nucleotiɗde

10   sequence as shown in SEQ ID NO:5.

20.   A method of screening for a compound that is capable of modulating the activity of a KCNQ protein, comprising the steps of:

    (a)   providing a host cell of claim 13;

    (b)   determining the activity of said KCNQ protein in the absence of ssaid

15   compound;

    (c)   contacting the cell with said compound; and

    (d)   determining the activity of said KCNQ protein in the presence of ʑsaid compound,

    wherein a difference between the activity of said KCNQ protein in the

20   presence of said compound and in the absence of said compound indicates a modulating compound.

21.   An anitbody specific for the KCNQ protein of claim 18.

22.   The antibody of claim 21, wherein said antibody is a monoclonal antibodďy.
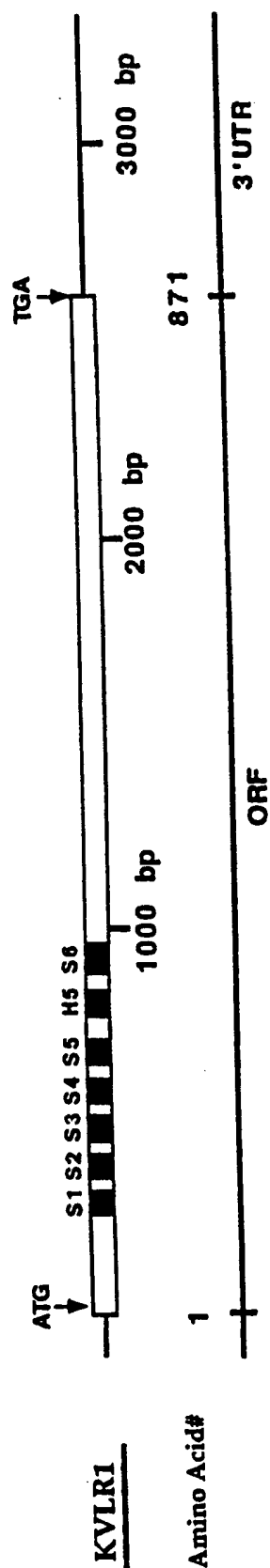
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FIG. 1

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CCCCCGCGCTCCGCCCCCGCTGAGCCTGAGCCCGACCGGGGCGCCTCCCGCCAGGCACCATGGTTGCAGAAGTCG
GGGGGCGCGAGGCGGGGGCGACTCGGACTCGGGCTGGGCCCCGCGGAGGGCGGTCCGTGGTACCACGTCTTCAGC
                                                        M  V  Q  K  S
```

```
                    100                                              150
CGCAACGGCGGCGTATACCCCGGCCCGAGCGGGGAGAAGAAGCTGAAGGTGGGCTTCGTGGGGCTTGGACCCCGGC
GCGTTGCCGCCGCATATGGGGCCGGGCTCGCCCCTCTTCTTCGACTTCCACCCGAAGCACCCCGAACCTGGGGCCG
 R  N  G  G  V  Y  P  G  P  S  G  E  K  K  L  K  V  G  F  V  G  IL  D  P  G>
```

```
                                        200
GCGCCCGACTCCACCCGGGACGGGGCGCTGCTGATCGCCGGCTCCGAGGCCCCCAAGCGCGGCAAGCATCCTCAGC
CGCGGGCTGAGGTGGGCCCTGCCCCGCGACGACTAGCGGCCGAGGCTCCGGGGGGTTCGCGCCGTTCGTAGGAGTCG
 A  P  D  S  T  R  D  G  A  L  L  I  A  G  S  E  A  P  K  R  G  IS  I  L  S
```

```
            250                                              300
AAACCTCGCGCGGGCGGCGCGGGCGCCGGGAAGCCCCCCAAGCGCAACGCCTTCTACCGCAAGCTGCAGAATTTC
TTTGGAGCGCGCCCGCCGCGCCCGCGGCCCTTCGGGGGGGTTCGCGTTGCGGAAGATGGCGTTCGAACGTCTTAAAG
 K  P  R  A  G  G  A  G  A  G  K  P  P  K  R  N  A  F  Y  R  K  IL  Q  N  F>
```

```
                                350
CTCTACAACGTGCTGGAGCGGCCGCGCGGCTGGGCGTTCATCTACCACGCCTACGTGTTCCTCCTGGTTTTTCTCC
GAGATGTTGCACGACCTCGCCGGCGCGCCGACCCGCAACTAGATGGTGCGGATGCACAAGGAGGAACCAAAAGAGG
 L  Y  N  V  L  E  R  P  R  G  W  A  F  I  Y  H  A  Y  V  F  L  IL  V  F  S
```

```
            400                                              450
TGCCTCGTGCTGTCTGTGTTTTTCCACCATCAAGGAGTATGAGAAGAGCTCGGAGGGGGCCCTCTTACATCCTGGAA
ACGGAGCACGACAGACACAAAAGGTGGTAGTTCCTCATACTCTTCTCGAGCCTCCCCCGGGGAGAATGTAGGACCTT
 C  L  V  L  S  V  F  S  T  I  K  E  Y  E  K  S  S  E  G  A  L  YI  L  E>
```

```
                                500
ATCGTGACTATCGTGGTGTTTGGCGTGGAGTACTTCGTGCGGATCTGGGCCGCAGGCTGCTGCTGCCGGTACCGT
TAGCACTGATAGCACCACAAACCGCACCTCATGAAGCACGCCTAGACCCGGCGTCCGACGACGAACGGCCATGGCA
 I  V  T  I  V  V  F  G  V  E  Y  F  V  R  I  W  A  A  G  C  C  IC  R  Y  R
```

```
            550                                              600
GGCTGGAGGGGGCGGCTCAAGTTTGCCCGGAAACCGTTCTGTGTGATTGACATCATGGTGCTCATCGCCTCCATT
CCGACCTCCCCCGCCGAGTTCAAACGGGCCTTTGGCAAGACACACTAACTGTAGTACCACGAGTAGCGGAGGTAA
 G  W  R  G  R  L  K  F  A  R  K  P  F  C  V  I  D  I  M  V  L  I  A  S  I>
```

FIG. 2A

650
GCGGTGCTGGCCGCCGGCTCCCAGGGCAACGTCTTTGCCACATCTGCGCTCCGGAGCCTGCGCTTTCCTGCAGATT
CGCCACGACCGGCGGCCGAGGGTCCCGTTGCAGAAACGGTGTAGACGCGAGGCCTCGGACGCGAAGGACGTCTAA
A V L A A G S Q G N V F A T S A L R S L R F L Q I

700   750
CTGCGGATGATCCGCATGGACCGGCGGGGAGGCACCTGGAAGCTGCTGGGCTCTGTGGTCTATCGCCCACAGCAAG
GACGCCTACTAGGCGTACCTGGCCGCCCCTCCGTGGACCTTCGACGACCCGAGACACCAGATAACGGGTGTCGTTC
L R M I R M D R R G G T W K L L G S V V Y A H S K>

800
GAGCTGGTCACTGCCTGGTACATCGGCTTCCTTTGTCTCATCCTGGCCTCGTTCCTGGTGTACTTTGGCAGAGAAG
CTCGACCAGTGACGGACCATGTAGCCGAAGGAAACAGAGTAGGACCGGAGCAAGGACCACATGAAACCGTCTCTTC
E L V T A W Y I G F L C L I L A S F L V Y L A E K

850   900
GGGGAGAACGACCACTTTGACACCTACGCGGATGCACTCTGGTGGGGCCTGATCACGCTGACCACCATTGGCTAC
CCCCTCTTGCTGGTGAAACTGTGGATGCGCCTACGTGAGACCACCCCGGACTAGTGCGACTGGTGGTAACCGATG
G E N D H F D T Y A D A L W W G L I T L T T I G Y>

950
GGGGACAAGTACCCCCAGACCTGGAACGGCAGGCTCCTTGCGGCAACCTTCACCCTCATCGGTGTCTCCTTCTTC
CCCCTGTTCATGGGGGTCTGGACCTTGCCGTCCGAGGAACGCCGTTGGAAGTGGGAGTAGCCACAGAGGAAGAAG
G D K Y P Q T W N G R L L A A T F T L I G V S F F

1000   1050
GCGCTGCCTGCAGGCATCTTGGGGGTCTGGGTTTGCCCTGAAGGTTCAGGAGCAGCACAGGCAGAAGCACTTTGAG
CGCGACGGACGTCCGTAGAACCCCAGACCCAAACGGGACTTCCAAGTCCTCGTCGTGTCCGTCTTCGTGAAACTC
A L P A G I L G S G F A L K V Q E Q H R Q K H F E>

1100
AAGAGGCGGAACCCGGCAGCAGGCCTGATCCAGTCGGCCTGGAGATTTTACGCCACCAACCTCTCGCGCACAGAC
TTCTCCGCCTTGGGCCGTCGTCCGGACTAGGTCAGCCGGACCTCTAAAATGCGGTGGTTGGAGAGCGCGTGTCTG
K R R N P A A G L I Q S A W R F Y A T N L S R T D

1150   1200
CTGCACTCCACGTGGCAGTACTACGAGCGAACGGTCACCGTGCCCATGTACAGTTCGCAAACTCAAACCTACGGG
GACGTGAGGTGCACCGTCATGATGCTCGCTTGCCAGTGGCACGGGTACATGTCAAGCGTTTGAAGTTTGGATGCCC
L H S T W Q Y Y E R T V T V P M Y S S Q T Q T Y G>

FIG. 2B

1250

```
GCCTCCAGACTTATCCCCCCGCTGAACCAGCTGGAGCTGCTGAGAAACCTCAAGAGTAAATCTGGGACTCGCTTTC
CGGAGGTCTGAATAGGGGGGCGACTTGGTCGACCTCGACGACTCTTTGGAGTTCTCATTTAGACCCTGAGCGAAAG
 A  S  R  L  I  P  P  L  N  Q  L  E  L  L  R  N  L  K  S  K  S  (G  L  A  F
```

1300   1350

```
AGGAAGGACCCCCCGCCGGAGCCGTCTCCAAGTAAAAGGCAGCCCGTGCAGAGGGCCCCTGTGTGGGATGCTGCCCC
TCCTTCCTGGGGGGCGGCCTCGGCAGAGGTTCATTTCCGTCGGGCACGTCTCCCGGGGACACACCCTACGACGGGG
 R  K  D  P  P  P  E  P  S  P  S  K  G  S  P  C  R  G  P  L  C  (G  C  C  P>
```

1400

```
GGACGCTCTAGCCAGAAGGTCAGTTTGAAAGATCGTGTCTTCTCCAGCCCCCGAGGCGTGGCTGGCCAAGGGGAAG
CCTGCGAGATCGGTCTTCCAGTCAAACTTTCTAGCACAGAAGAGGTCGGGGGGCTCCGCACCGACCGGTTCCCCTTC
 G  R  S  S  Q  K  V  S  L  K  D  R  V  F  S  S  P  R  G  V  A  /A  K  G  K
```

1450   1500

```
GGGTCCCCGCAGGCCCAGACTGTGAGGCGGTCACCCAGCGCCGACCAGAGCCTCGAGGACAGCCCCCAGCAAGGTG
CCCAGGGGCGTCCGGGTCTGACACTCCGCCAGTGGGTCGCGGCTGGTCTCGGAGCTCCTGTCGGGGGTCGTTCCAC
 G  S  P  Q  A  Q  T  V  R  R  S  P  S  A  D  Q  S  L  E  D  S  /P  S  K  V>
```

1550

```
CCCAAGAGCTGGAGCTTCGGGGACCGCAGCCGGGCACGCCAGGCTTTCCGCATCAAGGGGTGCGGGCGTCACGGCAG
GGGTTCTCGACCTCGAAGCCCCTGGCGTCGGCCCGTGCGGTCCGAAAGGCGTAGTTCCCACGCCCGCAGTGCCGTC
 P  K  S  W  S  F  G  D  R  S  R  A  R  Q  A  F  R  I  K  G  A  /A  S  R  Q
```

1600   1650

```
AACTCAGAAGCAAGCCTCCCCGGAGAGGACATTGTGGATGACAAGAGCTGCCCCTGCGAGTTTGTGACCGAGGAC
TTGAGTCTTCGTTCGGAGGGGCCTCTCCTGTAACACCTACTGTTCTGACGGGGACGCTCAAACACTGGCTCCTG
 N  S  E  A  S  L  P  G  E  D  I  V  D  D  K  S  C  P  C  E  F  /V  T  E  D>
```

FIG. 2C

1700
CTGACCCCGGGGCCTCAAAGTCAGCATCAGAGCCGTGTGTGTCATGCGGTTCCTGGTGTCCAAGCGGGAAGTTCAAG
GACTGGGGCCCGGAGTTTCAGTCGTAGTCTCGGCACACACAGTACGCCAAGGACCACAGGTTCGGCCTTCAAGTTC
L T P G L K V S I R A V C V M R F L V S K R K F K

                                   1750   1800
GAGAGCCTGCGGCCCTACGACGTGATGGACGTCATCGAGCAGTACTCAGCCGGCCACCTGGACAATGCTGTCCCGA
CTCTCGGACGCCGGGATGCTGCACTACCTGCAGTAGCTCGTCATGAGTCGGCCGGTGGACCTGTTACGACAGGGCT
E S L R P Y D V M D V I E Q Y S A G H L D M L S R>

                                   1850
ATTAAGAGCCTGCAGTCCAGAGTGGACCAGATCGTGGGGCGGGGCCCAGCGATCACGGACAAGGGACCGCACCAAG
TAATTCTCGGACGTCAGGTCTCACTGGTCTAGCACCCCGCCCCGGGTCGCTAGTGCCTGTTCCCTGGCGTGGTTC
I K S L Q S R V D Q I V G R G P A I T D K D R T K

                                   1900   1950
GGCCCGGCCGAGGCGGAGCTGCCCGAGGACCCCAGCATGATGGGACGGCTCGGGAAGGTGGAGAAAGCAGGTCTTG
CCGGGCCGGCTCCGCCTCGACGGGCTCCTGGGGTCGTACTACCCTGCCGAGCCCTTCCACCTCTTTCGTCCAGAAC
G P A E A E L P E D P S M M G R L G K V E K Q V L>

                                   2000
TCCATGGAGAAGAAGCTGGACTTCCTGGTGAATATCTACATGCAGCGGATGGGCATCCCCCCCGAACAGAGACCGAG
AGGTACCTCTTCTTCGACCTGAAGGACCACTTATAGATGTACGTCGCCTACCCGTAGGGGGGCTTGTCTCTGGCTC
S M E K K L D F L V N I Y M Q R M G I P P T E T E

                                   2050   2100
GCCTACTTTGGGGCCAAAGAGCCGGAGCCGGCGCCGCCGTACCACAGCCCGGAAGACAGCCGGGGAGCATGTCGAC
CGGATGAAACCCCGGTTTCTCGGCCTCGGCCGCGGCGGCATGGTGTCGGGCCTTCTGTCGGCCCCTCGTACAGCTG
A Y F G A K E P E P A P P Y H S P E D S R E H V D>

FIG. 2D

2150
AGGCACGGCTGCATTGTCAAGATCGTGCGCTCCAGCAGCTCCACGGGGCCAGAAGAACTTCTCGGGCGCCCCCGGCC
TCCGTGCCGACGTAACAGTTCTAGCACGCGAGGTCGTCGAGGTGCCCGGTCTTCTTGAAGAGCCCGCGGGGGGCCGG
R H G C I V K I V R S S S S T G Q K N F S A P P A

2200   2250
GCGCCCCCTGTCCAGTGTCCGCCCTCCACCTCCTGGCAGCCACAGAGCCACCCGCGCCAGGGCCCACGGCACCTCC
CGCGGGGGACAGGTCACAGGCGGGAGGTGGAGGACCGTCGGTGTCTCGGTGGGCGCGGTCCCGGGTGCCGTGGAGG
A P P V Q C P P S T S W Q P Q S H P R Q G   H G T S>

2300
CCCGTGGGGGACCACGGCTCCCTGGTGCGCATCCCGCCGCCGCCTGCCCACGAGCGGTCGCTGTTCCGCCTACGGC
GGGCACCCCCTGGTGCCGAGGGACCACGCGTAGGGCGGCGGCGGACGGGTGCTCGCCAGCGACAAGGCGGATGCCG
P V G D H G S L V R I P P P P A H E R S L S A Y G

2350   2400
GGGGGCAACCGCGCCAGCATGGAGTTCCTGCGGCAGGAGGACACCCCCGGGCTGCAGGCCCCCCGGAGGGGACCCTG
CCCCCGTTGGCGCGGTCGTACCTCAAGGACGCCGTCCTCCTGTGGGGGCCCGACGTCCGGGGGGGCCTCCCCTGGGAC
G G N R A S M E F L R Q E D T P G C R P P E G T L>

2450
CGGGACAGCGACACGTCCATCTCCATCCCGTCCGTGGACCACGAGGAGCTGGAGCGTTCCTTCAAGCGGCTTCAGC
GCCCTGTCGCTGTGCAGGTAGAGGTAGGGCAGGCACCTGGTGCTCCTCGACCTCGCAAGGAAGTTCGCCGAAGTCG
R D S D T S I S I P S V D H E E L E R S F S G F S

2500   2550
ATCTCCCAGTCCAAGGAGAACCTGGATGCTCTCAACAGCTGCTACGCGGCCGTGGCGCCCTTGTGGCCAAAGTCAGG
TAGAGGGTCAGGTTCCTCTTGGACCTACGAGAGTTGTCGACGATGCGCCGGCACCGCGGAACACCGGTTTCAGTCC
I S Q S K E N L D A L N S C Y A A V A P C A K V R>

2600
CCCTACATTGCGGAGGGAGAGTCAGACACCGACTCCGACCTCTGTACCCCGTGCGGGCCCCCGCCCACGCTCGGCC
GGGATGTAACGCCTCCCTCTCAGTCTGTGGCTGAGGCTGGAGACATGGGGCACGCCCGGGGGCGGGTGCGAGCCGG
P Y I A E G E S D T D S D L C T P C G P P P R S A

2650   2700
ACCGGCGAGGGTCCCTTTGGTGACGTGGGCTGGGCCGGGCCCAGGAAGTGAGGCGGCGCTGGGCCCAGTGGACCCG
TGGCCGCTCCCAGGGAAACCACTGCACCCGACCCGGCCCGGGTCCTTCACTCCGCCGCGACCCGGGTCACCTGGGC
T G E G P F G D V G W A G P R K>

FIG. 2E

```
                                        2750
CCCGCGGCCCTCCTCAGCACGGTGCCTCCGAGGTTTTGAGGCGGGAACCCTTTGGGGCCCTTTTCTTTACAGTAAC
GGGCGCCGGGAGGAGTCGTGCCACGGAGGCTCCAAAACTCCGCCCTTGGGAAACCCCGGGAAAAGAAATGTCATTG
                  2800                                            2850
TGAGTGTGGCGGGAAGGGTGGGCCCTGGAGGGGCCCATGTGGGCTGAAGGATGGGGGCTCCTGGCAAGTGACCTTT
ACTCACACCGCCCTTCCCACCCGGGACCTCCCCGGGTACACCCGACTTCCTACCCCCGAGGACCGTTCACTGGAAA
                                  2900
TACAAAAGTTATTTTTCCAACAGGGGCTGGAGGGCTGGGCAGGGCCCTGTGGCTCCAGGAGCAGCGTTGCAGGAGCA
ATGTTTTTCAATAAAAGGTTGTCCCCGACCTCCCGACCCGTCCCGGGACACCGAGGTCCTCGTCGCAACGTCCTCGT
                  2950                              •           3000
AGGCTGCCCTGTCCACTCTGCTCAGGGCCGCGGCCGACATCAGCCCGGTGTGAGGAGGGGCGGGAAGTGATGACGG
TCCGACGGGACAGTTGAGACGAGTCCCGGCGCCGGCTGTAGTCGGGCCACACTCCTCCCCGCCCTCCACTACTGCC
                                  3050
GGTGTTGCCAGCGTGGCAACAGGCGGGGGGGTTGTTTCAGCCGAGCCCAGGGGGAGGCACAAAGGGCAAGGCCTGTTC
CCACAACGGTCGCACCGTTGTCCGCCCCCCAACAAAGTCGGCTCGGGTCCCCTCCGTGTTTCCCGGTCCGGACAAG
                  3100                                          3150
CCTGAGGACCTGCGCAAAGGGCGGGCCTGTTTGGTGAGGACCTGCGGCCTTGGGTCCCGGTGGGGGTTTCCGGGCA
GGACTCCTGGACGCGTTTCCCGCCCGGACAAACCACTCCTGGACGCCGGAACCCAGGGCCACCCCCAAAGGCCCGT
                                  3200
GCTACAGGCGGGTGTGGCCGGCCGCTGTGCGTGGCCTCTGCCTTCACACCTGACCTGCCCGGCGGGGCTTTCCTGT
CGATGTCCGCCCACACCGGCCGGCGACACGCACCGGAGACGGAAGTGTGGACTGGACGGGCCGCCCCGAAAGGACA
                  3250
TCCCCACCTCAGGGGCGCCCAAATACAGAGCTATTGGTTGGCGTCTTAAAAAAAAAAAAAAAA
AGGGGTGGAGTCCCCGCGGGTTTATGTCTCGATAACCAACCGCAGAATTTTTTTTTTTTTTTT
```

FIG. 2F

KVLQT1 MAAASPPPRAERKRWGWGRLPGARRGSAGLAKKCPFSLELAEGGPAGGALYAPIA 55

KVLR1   MVQKSRNGGVYPGPSGEKKLKVGFVGLD 28

KVLQT1 PGAPGPAPPAS--PAAPAAPPVASDLGPRPPVSLDPRVSIYSTRRPVLARTHVQGRVYNFLERPTGWKCFVYHFAVF 130

KVLR1   PGAPDSTRDGALLIAGSEAPKRGSIL-SKPRAG-GAGAGK-PPKRNAFYR-KLQNFLYNVLERPRGWA-FIYHAYVF 100

         S1   S2   S3

KVLQT1 LIVLVCLIFSVLSTIEQYAALATGTLFWMEIVLVVFFGTEYVVRLWSAGCRSKYVGLWGRLRFARKPISIIDLIVVV 207

KVLR1   LLVFSCLVLSVFSTIKEYEKSSEGALYILEIVTIVVFGVEYFVRIWAAGCCCRYRGWRGRLKFARKPFCVIDIMVLI 177

         S4   S5

KVLQT1 ASMVVLCVGSKGQVFATSAIRGIRFLQILRMLHVDRQGGTWRLLGSVVFIHRQELITTLYIGFLIFSSYFVYLAE 284

KVLR1   ASIAVLAAGSQGNVFATSALRSLRFLQILRMIRMDRRGGTWKLLGSVVYAHSKELVTAWYIGFLCLILASFLVYLAE 254

        Pore   S6

KVLQT1 KDAVNESGRVEFGSYADALWWGVVTVTTIGYGDKVPQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQQKQRQ 361

KVLR1   K---GENDH--FDTYADALWWGLITLTTIGYGDKYPQTWNGRLLAATFTLIGVSFFALPAGILGSGFALKVQEQHRQ 326

KVLQT1 ...RAAASLIQTAWRCYAAE--NPD-SSTWKIYIRKAPRSHTLLSPSPKPKKSVVVKKKKFKLDKDNG// 430

KVLR1   KHFEKRRNPAAGLIQSAWRFYATNLSRTDLHSTWQYYERTVTVPMYS-SQTQTYGASRLIPPLNQLELLRNL// 398

FIG. 3

KvLQT1   MAAASSPPRAERKRWGWGRLPGARRGSAGLAKKCPFSLELAEGGPAGGALYAPIA   55
KvLR2   PAGGDAAAAGDEERKVGLAPGDVEQVTLA   29

                                        S1
KvLQT1   PGAPGPAPPAS--PAAPAAPPVASDLGPRPPVSLDPRVSIYSTRRPVLARTHVQGRVYNFLERPTGWKCFVYHFAVF   130
KvLR2   LGA-GADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYR-RIQTLIYDALERPRGWALL-YHALVF   103

              S2   S3
KvLQT1   LIVLVCLIFSVLSTIEQYAALATGTLFWMEIVLVVFFGTEYVVRLWSAGCRSKYVGLWGRLRFARKPISIIDLIVVV   207
KvLR2   LIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGCCCRYKGWRGRLKFARKPLCMLDIFVLI   180

              S4   S5
KvLQT1   ASMVVLCVGSKGQVFATSAIRGIRFLQILRMLHVDRQGGTWRLLGSVVFIHRQELITTLYIGFLGLIFSSYFVYLAE   284
KvLR2   ASVPVVAVGNQGNVLATSL--RSLRFLQILRMLRMDRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVE   256

                    Pore   S6
KvLQT1   KD-----AVNESGRVEFGSYADALWWGVVTVTIGYGDKVPQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQ   356
KvLR2   KDVPEVDAQGEEMKEEFETYADALWWGLITLATIGYGDKTPKIWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ   333

KvLQT1   QKQRQKHFN-RQIPAAASLIQTAWRCYAAENPDSSTW//   392
KvLR2   EQHRQKHFEKRRKPAAE-LIQAAWRYYAT-NPNRIDLVGDM//   372

FIG. 4

Putamen
Temporal Lobe
Frontal Lobe
Occipital Lobe
Spinal Cord
Medulla
Cerebral Cortex
Cerebellum

Thalamus
Subthalamic Nucleus
Substania Nigra
Total Brain
Hippocampus
Corpus Callosum
Caudate Nucleus
Amygdala

Pancreas
Kidney
Skeletal Muscle
Liver
Lung
Placenta
Brain
Heart

Kb 9.5 - 7.5 - 4.4 - 2.4 - 1.3 -   FIG. 5A

Kb 9.5 - 7.5 - 4.4 - 2.4 - 1.3 -   FIG. 5B

FIG. 6A

FIG. 6B

FIG. 6C

FIG. 6D
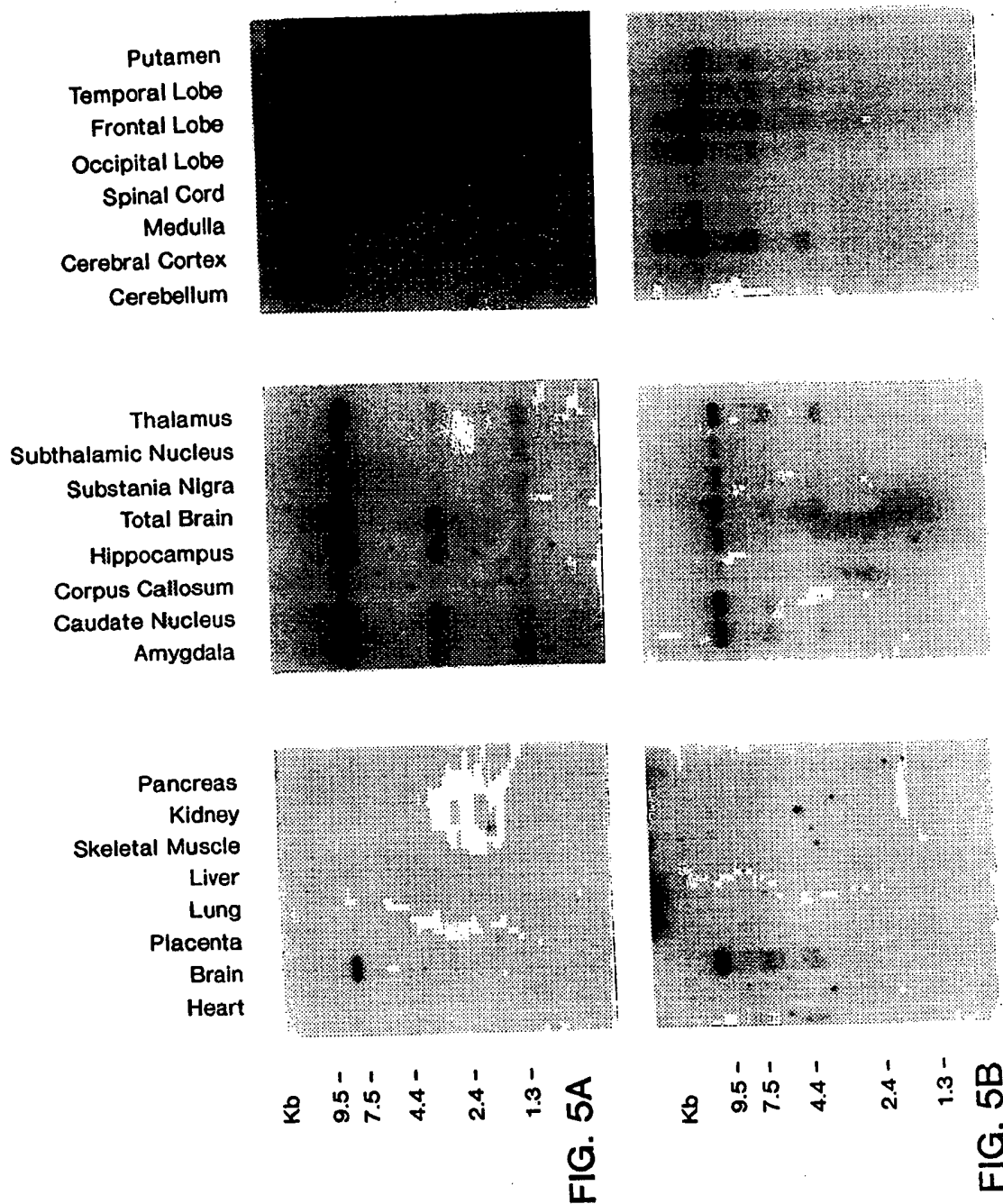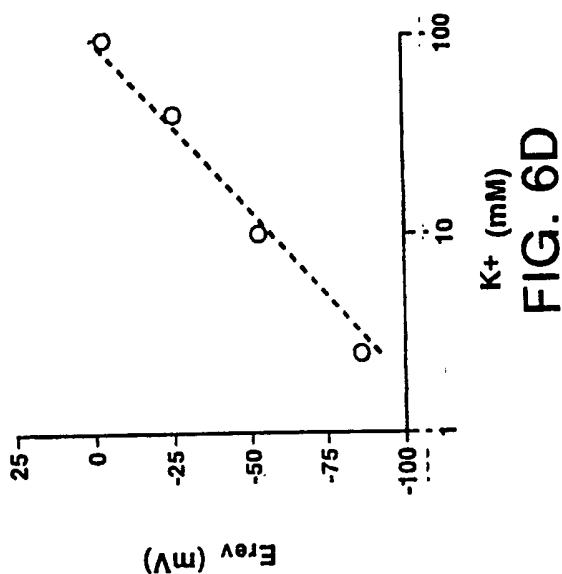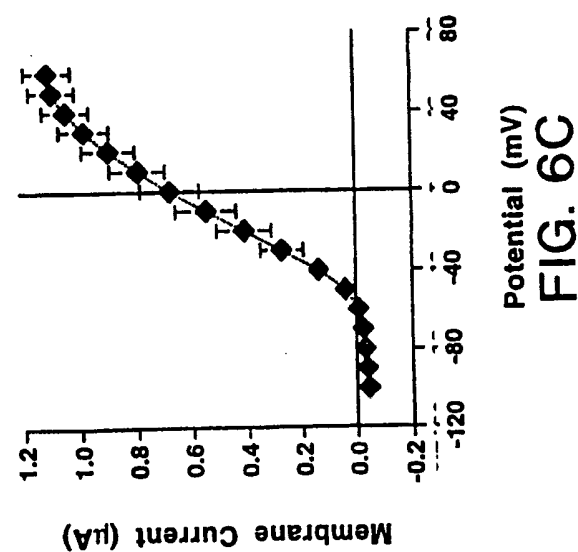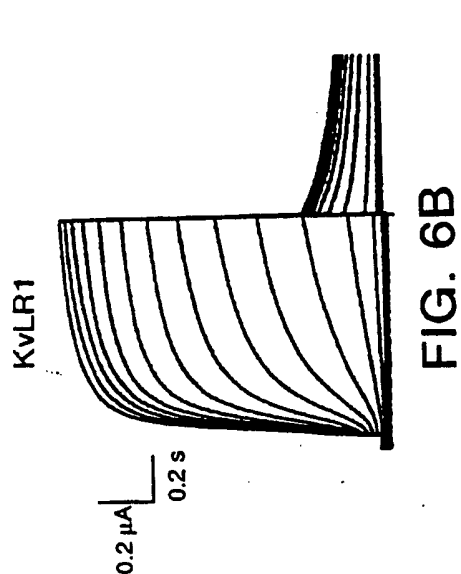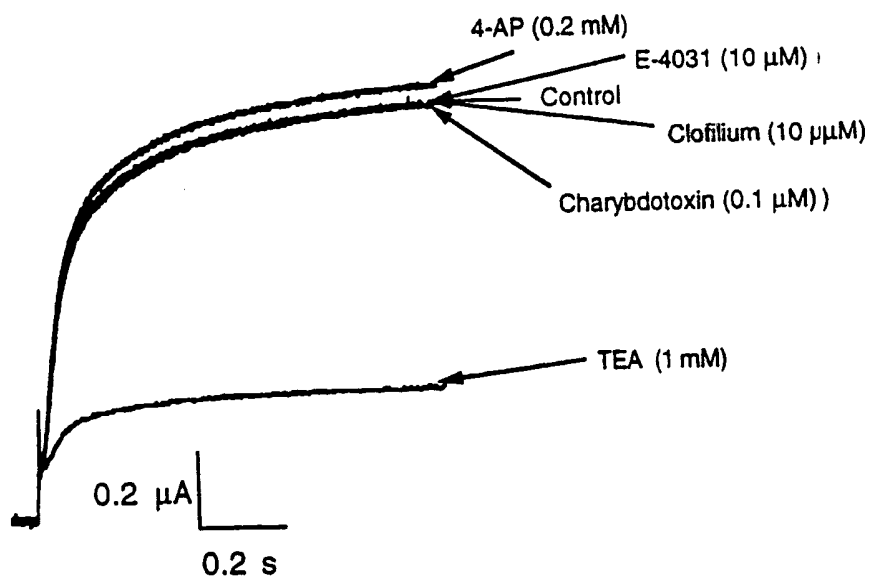
FIG. 7
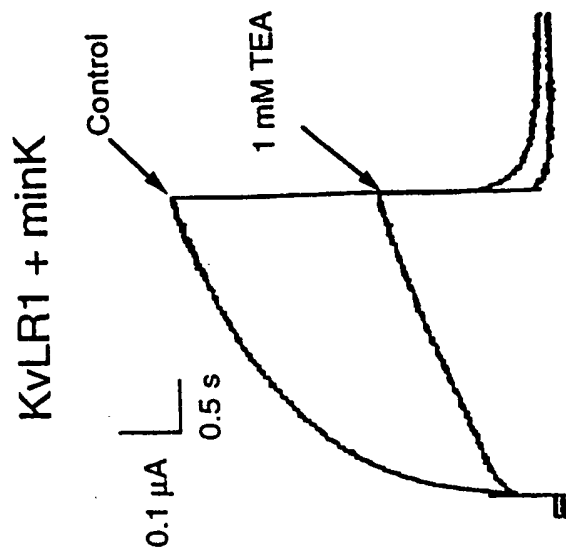
FIG. 8B
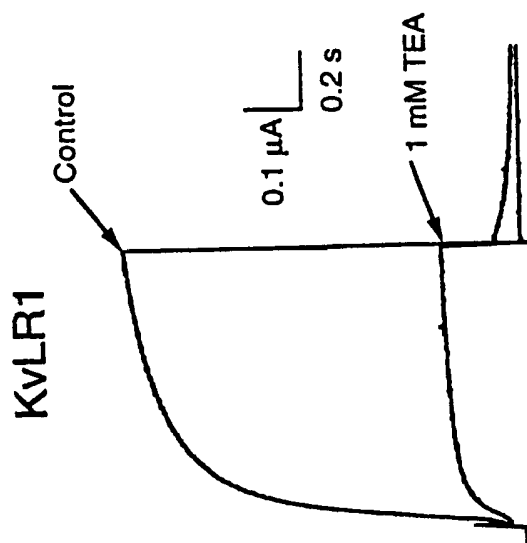
FIG. 8A

FIG. 9A

FIG. 9B

```
                                                                        90
  30                              60                                      *
   *                              *
GAGCAGCACAGGCAGAGAAACACTTTGAGAAACGGGCGGAACCCTGCGGCCAGGTCTGATCCAGTCTGCCTGGAGATTCTATGCTACTAACCTC
CTCGTCGTGTCCGTCTTTGTGAAACTCTTTGCCGCCCTTGGGACGCCGGTCAGACTAGGTCAGACGAACGATGATTGGAG
E  Q  H  R  Q  K  H  F  E  K  R  R  N  P  A  A  G  L  I  Q  S  A  W  R  F  Y  A  T  N  L>

                              150                                      180
  120                          *                                        *
   *
TTACGCCACCGACCTGCACTCCACGTGGCAGTACTACGAGCGGACAGTCACTGTCCCCATGTACAGCTCAAACTCAAACCTATGGGGCC
AATGCGGTGGCTGGACGTGGAGGTGCACCGTGCTCGCCGTCTCAGTGACAGGGTACATGTCGAGTGTTTGGATACCCCGG
L  R  T  D  L  H  S  T  W  Q  Y  Y  E  R  T  V  T  V  P  M  Y  S  S  Q  T  Q  T  Y  G  A>

  210
   *
TCCAGACTCATCCCACCTCTGAACCAGCTGGAGCTGCTGAGAAACA
AGGTCTGAGTAGGGTGGAGACTTGGTCGACCTCGACGACTCTTTGT
S  R  L  I  P  P  L  N  Q  L  E  L  L  R  N>
```

FIG. 9C

```
ATG GTG CAG AAG TCG CGC AAC GGT GGC GTG TAC CCC GGC ACC  AGC 45
 M   V   Q   K   S   R   N   G   G   V   Y   P   G   T    S
             5                  10                      15

GGG GAA AAG AAG CTC AAG GTG GGC TTC GTG GGG CTG GAC CCC GGC 90
 G   E   K   K   L   K   V   G   F   V   G   L   D   P   G
                20              25                      30

GCG CCC GAC TCC ACA CGC GAC GGC GCG CTA CTC ATC GCG GGC TCC 135
 A   P   D   S   T   R   D   G   A   L   L   I   A   G   S
                35              40                      45

GAG GCC CCC AAG CGC GGC AGC GTT TTG AGC AAG CCG CGG ACG GGC 180
 E   A   P   K   R   G   S   V   L   S   K   P   R   T   G
                50              55                      60

GGC GCG GGA GCC GGG AAG CCC CCG AAG CGC AAC GCC TTC TAC CGC 225
 G   A   G   A   G   K   P   P   K   R   N   A   F   Y   R
                65              70                      75

AAG CTG CAG AAT TTC CTC TAC AAC GTG CTA GAG CGG CCC CGC  GGC 270
 K   L   Q   N   F   L   Y   N   V   L   E   R   P   R   G
                80              85                      90

TGG GCG TTC ATC TAC CAC GCC TAC GTG TTC CTT TTA GTC TTC  TCC 315
 W   A   F   I   Y   H   A   Y   V   F   L   L   V   F   S
                95              100                     105

TGC CTT GTG CTT TCT GTG TTT TCC ACC ATC AAG GAG TAC GAG  AAG 360
 C   L   V   L   S   V   F   S   T   I   K   E   Y   E   K
                110             115                     120

AGC TCT GAG GGG GCC CTC TAC ATC TTG GAA ATC GTG ACT ATC  GTG 405
 S   S   E   G   A   L   Y   I   L   E   I   V   T   I   V
                125             130                     135

GTA TTC GGT GTT GAG TAC TTT GTG AGG ATC TGG GCT GCA GGC  TGC 450
 V   F   G   V   E   Y   F   V   R   I   W   A   A   G   C
                140             145                     150

TGT TGC CGG TAT CGA GGC TGG AGG GGC AGG CTC AAG TTT GCC  AGG 495
 C   C   R   Y   R   G   W   R   G   R   L   K   F   A   R
                155             160                     165

AAG CCG TTC TGT GTG ATT GAT ATC ATG GTG CTG ATT GCC TCC  ATT 540
 K   P   F   C   V   I   D   I   M   V   L   I   A   S   I
                170             175                     180

GCT GTG CTG GCT GCT GGT TCC CAG GGC AAT GTC TTT GCC ACA  TCT 585
 A   V   L   A   A   G   S   Q   G   N   V   F   A   T   S
                185             190                     195
```

FIG. 10A

GCG CTT CGG AGC TTG CGG TTC TTG CAA ATC TTG CGG ATG ATCC CGT 630
A   L   R   S   L   R   F   L   Q   I   L   R   M   I   R   210
                    200   205

ATG GAC CGG AGG GGT GGC ACC TGG AAG CTC TTG GGA TCG GTPA GTC 675
M   D   R   R   G   G   T   W   K   L   L   G   S   V   V   225
                    215   220

TAC GCT CAC AGC AAG GAG CTG GTG ACT GCC TGG TAC ATT GGCC TTC 720
Y   A   H   S   K   E   L   V   T   A   W   Y   I   G   F   240
                    230   235

CTC TGC CTC ATC CTG GCC TCA TTT CTG GTG TAC TTG GCA GAAA AAG 765
L   C   L   I   L   A   S   F   L   V   Y   L   A   E   K   255
                    245   250

GGT GAG AAT GAC CAC TTT GAC ACC TAC GCA GAT GCA CTC TGCG TGG 810
G   E   N   D   H   F   D   T   Y   A   D   A   L   WI   W   270
                    260   265

GGT CTG ATC ACC CTG ACG ACC ATT GGC TAC GGG GAC AAG TACC CCT 855
G   L   I   T   L   T   T   I   G   Y   G   D   K   Y   P   285
                    275   280

CAG ACC TGG AAC GGG AGG CTG CTG GCA GCG ACC TTT ACC CTCC ATT 900
Q   T   W   N   G   R   L   L   A   A   T   F   T   L   I   300
                    290   295

GGT GTC TCG TTC TTT GCT CTT CCT GCT GGC ATT TTG GGA TCCC GGC 945
G   V   S   F   F   A   L   P   A   G   I   L   G   S   G   315
                    305   310

TTT GCC CTG AAA GTC CAA GAG CAG CAT CGG CAA AAA CAC TTTT GAG 990
F   A   L   K   V   Q   E   Q   H   R   Q   K   H   F   E   330
                    320   325

AAA CGG CGG AAC CCT GCG GCA GGT CTG ATC CAG TCT GCC TGGG AGA 1035
K   R   R   N   P   A   A   G   L   I   Q   S   A   WI   R   345
                    335   340

TTC TAT GCT ACT AAC CTC TCA CGC ACC GAC CTG CAC TCC ACCG TGG 1080
F   Y   A   T   N   L   S   R   T   D   L   H   S   TP   W   360
                    350   355

CAG TAC TAC GAG CGG ACA GTC ACT GTC CCC ATG TAC AGA CTCC ATC 1125
Q   Y   Y   E   R   T   V   T   V   P   M   Y   R   LI   I   375
                    365   370

CCA CCT CTG AAC CAG CTG GAG CTG CTG AGG AAT CTC AAG AGGC AAA 1170
P   P   L   N   Q   L   E   L   L   R   N   L   K   SI   K   390
                    380   385

FIG. 10B

```
TCT GGA CTC ACC TTC AGG AAG GAG CCA CAG CCA GAG CCA TCA: CCA 1215
 S   G   L   T   F   R   K   E   P   Q   P   E   P   S    P
            395                 400                 405

AGT CAG AAG GTC AGT TTG AAA GAT CGT GTC TTC TCC AGC CCC: CGA 1260
 S   Q   K   V   S   L   K   D   R   V   F   S   S   P    R
            410                 415                 420

GGC ATG GCT GCC AAG GGA AAG GGG TCT CCC CAG GCC CAG ACG; GTC 1305
 G   M   A   A   K   G   K   G   S   P   Q   A   Q   T    V
            425                 430                 435

CGG CGG TCC CCC AGT GCG GAT CAG AGT CTT GAT GAC AGC CCG; AGC 1350
 R   R   S   P   S   A   D   Q   S   L   D   D   S   P    S
            440                 445                 450

AAG GTG CCC AAG AGC TGG AGC TTT GGT GAC CGC AGC CGC ACA\ CGC 1395
 K   V   P   K   S   W   S   F   G   D   R   S   R   T    R
            455                 460                 465

CAG GCT TTC CGC ATC AAG GGT GCT GCA TCC CGG CAG AAT TCA\ GAA 1440
 Q   A   F   R   I   K   G   A   A   S   R   Q   N   S    E
            470                 475                 480

GCA AGC CTC CCT GGG GAG GAC ATC GTA GAG GAC AAC AAG AGC: TGT 1485
 A   S   L   P   G   E   D   I   V   E   D   N   K   S    C
            485                 490                 495

AAC TGC GAG TTT GTG ACT GAA GAT CTT ACC CCT GGC CTC AAA\ GTT 1530
 N   C   E   F   V   T   E   D   L   T   P   G   L   K    V
            500                 505                 510

AGC ATC AGA GCT GTG TGT GTT ATG CGG TTC TTG GTA TCT AAG; CGA 1575
 S   I   R   A   V   C   V   M   R   F   L   V   S   K    R
            515                 520                 525   .

AAG TTC AAA GAG AGT CTG CGC CCA TAT GAT GTG ATG GAC GTC: ATC 1620
 K   F   K   E   S   L   R   P   Y   D   V   M   D   V    I
            530                 535                 540

GAA CAG TAC TCG GCT GGA CAC TTG GAT ATG TTG TCC CGC ATC: AAG 1665
 E   Q   Y   S   A   G   H   L   D   M   L   S   R   I    K
            545                 550                 555

AGC CTG CAG TCC AGA GTG GAC CAG ATT GTG GGG CGG GGC CCA\ ACA 1710
 S   L   Q   S   R   V   D   Q   I   V   G   R   G   P    T
            560                 565                 570

ATA ACG GAT AAG GAT CGC ACC AAA GGC CCA GCG GAA ACG GAG; CTG 1755
 I   T   D   K   D   R   T   K   G   P   A   E   T   E    L
            575                 580                 585
```

FIG. 10C

```
CCC GAA GAC CCC AGC ATG ATG GGA CGG CTT GGG AAG GTG GAG: AAA 1800
 P   E   D   P   S   M   M   G   R   L   G   K   V   E    K
                590                 595                     600

CAG GTC TTG TCC ATG GAA AAG AAG CTC GAC TTC TTG GTG AGC: ATC 1845
 Q   V   L   S   M   E   K   K   L   D   F   L   V   S    I
                605                 610                     615

TAT ACA CAG AGA ATG GGC ATC CCA CCA GCA GAG ACA GAG GCC: TAT 1890
 Y   T   Q   R   M   G   I   P   P   A   E   T   E   A    Y
                620                 625                     630

TTT GGG GCC AAG GAG CCT GAG CCG GCA CCA CCC TAC CAC AGC: CCA 1935
 F   G   A   K   E   P   E   P   A   P   P   Y   H   S    P
                635                 640                     645

GAG GAC AGC CGT GAC CAT GCA GAC AAG CAT GGC TGT ATC ATT: AAG 1980
 E   D   S   R   D   H   A   D   K   H   G   C   I   I    K
                650                 655                     660

ATC GTC CGC TCC ACC AGC TCT ACG GGC CAG AGG AAC TAC GCA\ GCA 2025
 I   V   R   S   T   S   S   T   G   Q   R   N   Y   A    A
                665                 670                     675

CCC CCA GCC ATC CCC CCT GCC CAG TGT CCT CCC TCC ACC TCG: TGG 2070
 P   P   A   I   P   P   A   Q   C   P   P   S   T   S    W
                680                 685                     690

CAG CAG AGC CAC CAG CGC CAT GGC ACC TCC CCT GTG GGA GAC: CAT 2115
 Q   Q   S   H   Q   R   H   G   T   S   P   V   G   D    H
                695                 700                     705

GGC TCA CTG GTC CTG CGA CTG GAG AGG AGT GCT GGC ATG ATG: AGC 2160
 G   S   L   V   L   R   L   E   R   S   A   G   M   M    S
                710                 715                     720

                                                          2169
TGT CAC TAG
 C   H   *
```
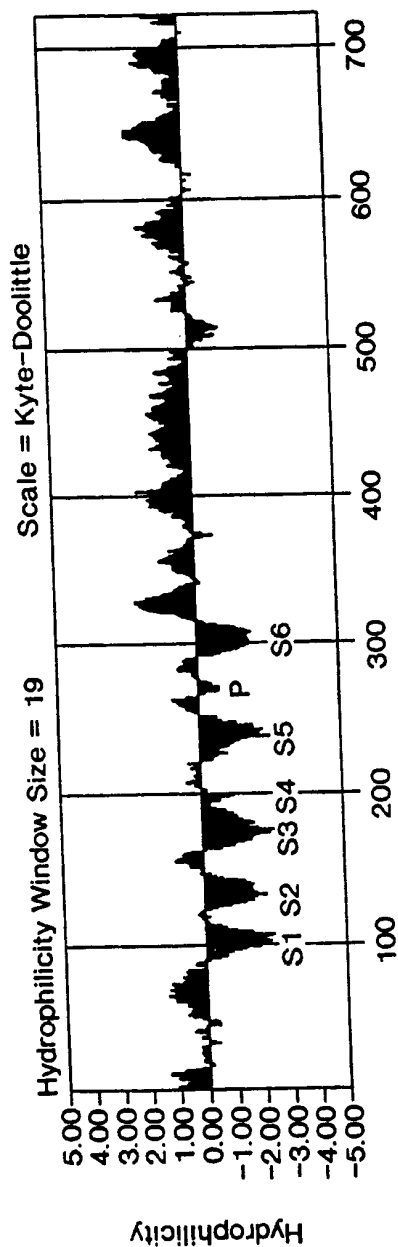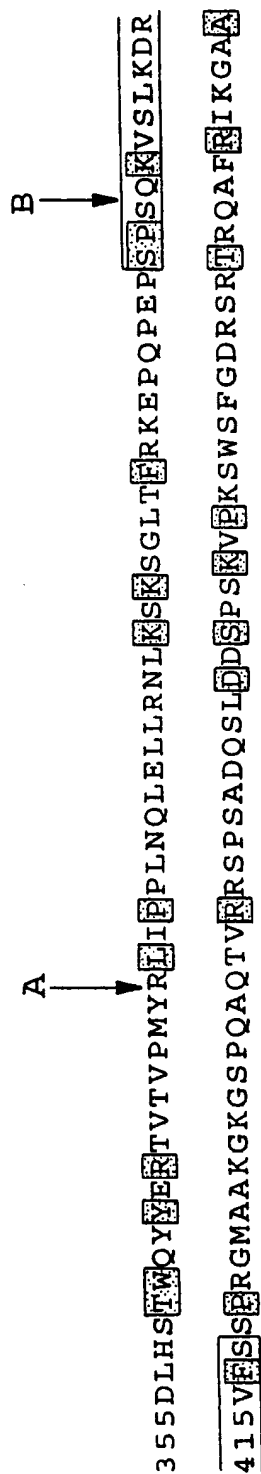
FIG. 10D

FIG. 10E

FIG. 11

brainKvLQT-R
heartKvLQT

```
brainKvLQT-R    1  MVQKSRNGGVYPGTSGEKKLKVGFVGLDPGAPDSTRDGALLIAGSEAPKRGSVLSKPRTG
heartKvLQT      1  M-----STPVSPAPADLGPRPRVSLDPRV-------------------SIYSARRPLLARTHIQ

brainKvLQT-R   61  GAGAGKPPKRNAFYRKLQNFLYNVLERPRGWA-FIYHAYVFLLVFSCLVLSVFSTIKEYE
heartKvLQT     43  GR----------------VYNFLERPTGWKCFVEVYHFTVFLIVLVCLIFSVLSTIEQYA

                                                            S1

brainKvLQT-R  120  KSSEGALYILEIVTIVVFGVEYFVRIWAAGCCCRYRGWRGRLKFARKPFCVTDIMVLIAS
heartKvLQT     85  ALATGTLFWMETLVLVVFGETEYVVRLWSAGCRSKYVGIWGRLRFARKPISILDLIVVAS

                        S2                                              S3

brainKvLQT-R  180  IAVLAAGSQGNVFATSALRSLRFLQILRMILRMDRRGGTWKLLGSVVYAHSKELVTAWYIG
heartKvLQT    145  MVVLCVGSKGQVFATSAIRGIRFLQILRMLHVDRQGGTWRLLGSVVFIHRQELITTLYIG

                               S4                                 Pore

brainKvLQT-R  240  FLCLILASFLVYLAEKGENDH----FDTYADALWWGLITLTTIGYGDKYPQTWNGRLLA
heartKvLQT    205  FLGLIFSSYFVYLAEKDAVNESGRIEEGSYADALWWGVITTLIGYGDKVPQTWNGKTIA

                    S5                                                S6

brainKvLQT-R  295  ATFTLIGVSFFALPAGILGSGFALKVQEQHRQKHFEKRRNPAAGLIQSAWRFYATNLSRT
heartKvLQT    265  SCFSVFAISFFALPAGILGSGFALKVQEQHRQKHFNRQIPAAASLIQTAWRCYAAENPDS

brainKvLQT-R  355  DLHSTWQYYERTVTVPMYRLIPLNQELELLRNLKSGLTERKEPQPEPSPSQKVSLKDR
heartKvLQT    325  -----ATWKIYVRKPARSHTLLSPKPKKSVMVKKKK-----EKLDKDNGMSPGEKM---

brainKvLQT-R  415  VFSSPRGMAAKGKGSPQAQTVRRSPSADQSLDDSPSKVPKSWSFGDRSRTRQAFRIKGAA
heartKvLQT    373  -ENVPH-------ITYDPPEDRRPDHFSIDGYDSSVRKSPTLLELS----IPHFLRTNSFA

brainKvLQT-R  475  SRQNSEASLPGEDIVEDNKSCNCEFVTEDLTPGLKVSIRAVCVMRFLVSKRKFKESLRPY
heartKvLQT    422  -----EDLDLEGETLLTPITH----VSQLRDHHRATIKVIRRMQYFVAKKKEQQARKPY

brainKvLQT-R  535  DVMDVIEQYSAGHLDMLSRIKSLQSRVDQIVGRGP----THTDKDRTKGPAETELPEDPSM
heartKvLQT    472  DVRDVIEQYSQGHLNLMVRIKELQRRLDQSIGKPSLFIPHSEKSKDRGSNTIG-------

brainKvLQT-R  592  MGRLGKVEKQVLSMEKKLDELVSIYTQRMGIPPAAETEAYFGAKEPEPAPYHSPEDSRDH----Q
heartKvLQT    525  -ARLNRVEDKVTQLDQRLVIITDMLHQLLSM------------------SINPELFLPSNSLPTYEQLTVPQTGPDEGS

brainKvLQT-R  652  ADKHGCIIKIVRSTSSTGQRNYAAPPAIPPAQCPPSTSWQQSHQRHGTSPVGDHGSLVLR
heartKvLQT    556

brainKvLQT-R  712  LERSAGMMSCH
heartKvLQT    605  -------S
```

SUBSTITUTE SHEET (RULE 26)

Splice Variant Analysis

355 DLHST W QYYE R TVTVPMYR L I P PLNQELLRNL K SK SGLT E RKEPQPEP S P SQ K VSLKDR

← A

B →

415 VF SS R RGMAAKGKGSPQAQTV R RSPSADQSL D S P S KV P KSWSFGDRSR T RQAF R IKGA A
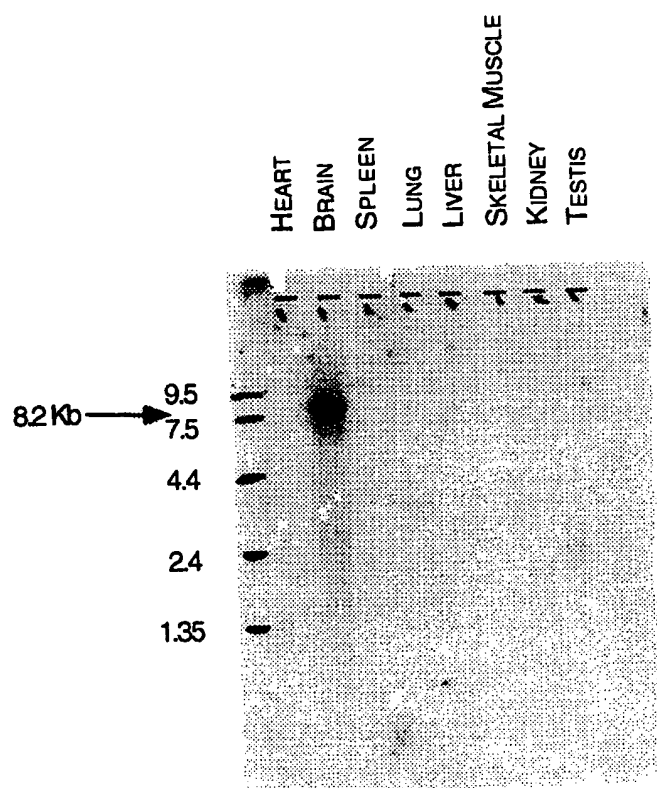
A. S S Q T Q T Y G A S
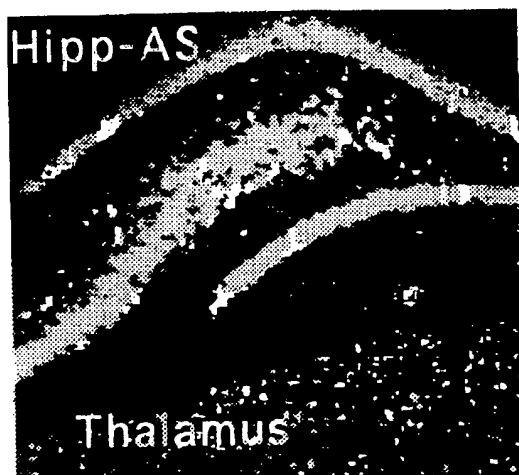
B. K G S P C R G P L C G C C P G R S S

FIG. 12

FIG. 13

FIG. 14A   FIG. 14B
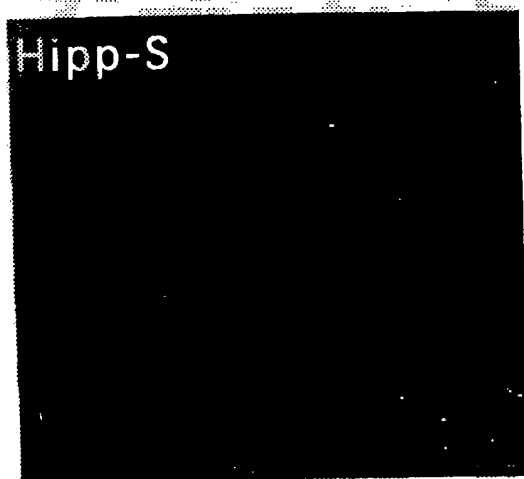

FIG. 14C   FIG. 14D

FIG. 15A

FIG. 15B

FIG. 16A

Consensus
hKvLR2
hKvLR1
mKvLR1

Consensus
hKvLR2
hKvLR1
mKvLR1

Consensus
hKvLR2
hKvLR1
mKvLR1
rKvLR1

Consensus
hKvLR2
hKvLR1
mKvLR1
rKvLR1

FIG. 16B

```
Consensus  -G-CT-AAGTTTGCC-GGAA-CC-CT-TG--TG-T-GA-ATC-T-GTGCT-AT-GCCTC--T--C-GTG-T-GC-
hKvLR2     CGACTGAAGTTTGCCAGGAAGAGCCCCTGTGCATGTTGGACATCTTTGTGCTGATTGCCTCTGTGCCCAGTGGTTGCT
             | | |||||||  ||   | |  |  ||   |     |||||    ||||||||   |  ||| |||
hKvLR1     CGGCTCAAGTTTGCCCGGAAACCGCTCTGTGTGATTGACATCATCGGCCTCATCGCCTCCATTGCGGTGCTGGCC
           |||||||||||||     |||||  || |||| ||||||||||  |||  || ||||||| |||||| |
mKvLR1     AGGCTCAAGTTTGCCAGGAAGAGCCGGTTCTGTGGTTCTGTGGTTCCATTGCCTCCATTGCTGTGCTGGCT
           ||||||||||||||  |||||| ||| |||||| |||| |  || ||||||||||| |||||||||||
rKvLR1     CGGCTCAAGTTTGCCAGGAAGAGCCATTCTGTGTGATCGACATCATGGTGCTGCTGATTGCCTCATTGCTGTGCTGGCT

Consensus  G---GG---CCA-GGCAA-GT--T-GC-AC-TC-***CT-CG-AGC-TGCG-TTC-T-CA-AT--T-CG-ATG-T-
hKvLR2     GTGGGAAACCAAGGCAATGTTCTGGCCACCTCC***CTGCGGAAGCCTGCGCTTCCTGCAGATCCTGCGCCATGCTG
             || | ||||||||||||| | |||||||||      || || || |||| ||||| |||||||| || |
hKvLR1     GCCGGCTCCCAGGGCAACGTCTTTGCCACATCTGCGCTCTGCGCTTCCTGCAGATTCTGCGGATGATC
           ||| ||||||||||||||| || ||||||||| | ||| ||||||||| |||||| |||||||||
mKvLR1     GCTGGTTCCCAGGGCAATGTCTTTGCCACATCTCTTGCCAAATCTTGCGGATGATC
           |||||||||||||||||| | ||||||||| |||| |||||||||||||| |||||
rKvLR1     GCTGGCTCCCAGGGCAATGTCTTTGCTACGTCTGCACTTCGGGTTCTTGCGGTTCTTACAAATCTTACGGATGATC

Consensus  CG-ATGGACCGG-G-GG-GGCACCTGGAAGCT--TGGG-TC-G--TCT--GC-CACAGCAA-GA-CT--T-AC-
hKvLR2     CGGATGGACCGGAGGAGTGGCACCTGGAAGCTTCTGGGCTCCTGGGCTCAGCCATCGTGCCCACAGCAAAGAACTCATCACG
           ||| |||||||| | || |||||||||||||| ||||| || |||||| ||| || | |||||||||| ||||| |||
hKvLR1     CGCATGGACCGGCGGGGAGGCACCTGGAAGCTGGAGCTCTGGGTCTGGGTCCTATGCCCACAGCTCACAGCAAGGAGCTGGTCACT
           ||||| ||||| ||| | ||||||||||||| ||| |||||  | | |||| |||| ||||||||||||||| |||
mKvLR1     CGTATGGACCGGAGGGGTGGCACCTGGAAGCTGGAGCTCTGGGATCGGGTAGTCTGCACGCTACGCGTCACAGCAAGGAGCTGGTGACT
           ||||| ||||||||| ||||||||||||||||||||| ||||| ||||| || ||||||||||||||
rKvLR1     CGTATGGACCGGAGGGGGGGCACCTGGAAGCTGGAGCTCTGGGATCGGGTCCTACGGTCACAGCAAGGAGCTGGTGACT

Consensus  GC TGGTACAT GG TTCCT     CTCATCCT   C TC TT CT GT TAC TGG  GA AA ****** GAG
hKvLR2     GCCTGGTACATCGGTTTCCTGACACTCATCCTTTCTCATTCTTGTCTACCTGGTTGAGAAAGACGTCCCAGAG
           |||||||||| ||||| ||||  || |||||| | || ||||  |  || ||||| |||||| ||  ||| |
hKvLR1     GCCTGGTACATTGGCTTCCTTTGTCTCATCCTGCCCTCATCCTGGTACTTGGCAGAGAAG*****GGGGAG
           ||||||||||| ||| ||||| | ||||||||| |||||||| | || |||||||||||    || |||
mKvLR1     GCCTGGTACATTGGCTTCCTGTGTCTGCCCTCATTCTGGTACTTGGCAGAAAG*****GGTGAG
           |||||||||| || |||||| | ||  | |||| ||  | ||||| ||||||    ||||||
rKvLR1     GCGTGGTACATTGGCTTCCTGTGTCTGCCCTCATTCTGGTACTTGGTACTTGGCAGAAAG*****GGTGAG
```

FIG. 16C

Consensus ---GA----***************************TT-GA-ACCTA-GC-GATGC-CT-TGGTGGGG-CTGATCAC-
hKvLR2   GTGGATGCACAAGGAGAGGAGATGAAAGAGGAGTTTGAGACCTATGCAGATGCCCTGTGGTGGGGCCTGATCACA
hKvLR1   AACGACCAC*******************TTTGACACCTACGCGGATGCACTCTGGTGGGGCCTGATCACG
mKvLR1   AATGACCAC*******************TTTGACACCTACGCAGATGCACTCTGGTGGGGTCTGATCACC
rKvLR1   AATGACCAC*******************TTCGACACCTACGCGGATGCACTCTGGTGGGGTCTGATCACC

Consensus CTG-C-ACCATTGGCTA-GG-GACAAG---CC--A-AC-TGG-A-GG--G-CT--T-GC-GC-AC-TT--CC-T-
hKvLR2   CTGGCCACCATTGGCTATGGAGACAAGACCACCCAAAACGTGGGAAGGCCGTCTGATTGCCGCCACCTTTCCTTA
hKvLR1   CTGACCACCATTGGCTACGGGGACAAGTACCCCAGACCTGGAACGGCAGGCTCCTTGCGGCAACCTTCACCCTC
mKvLR1   CTGACGACCATTGGCTACGGGGACAAGTACCCCAGACCTGGAACGGGAGGCTGCTGGCAGCGACCTTTACCCTC
rKvLR1   CTGACAACCATTGGCTACGGGGACAAGTACCCCAGACCTGGAACGGGAGGCTGTTAGCAGCGACGTTTACCCTC

Consensus AT-GG-GTCTC-TT-TT-GC-CT-CC-GC-GGCAT--TGGG-TC-GG--T-GCCCT-AA-GT-CA-GAGCA-CA-
hKvLR1   ATTGGCGTCTCCTTTTTGCCCTTCCAGCGGGCATCCTGGGCTCCGGGTCCGGGCTGGCCCTCAAGGTGCAGGAGCAACAC
mKvLR1   ATCGGTGTCTCTTCTTCGCGCTGCTTCCTGCAGGCATCTTGGGTCTGGGTTGCCCTGAAGGTTCAGGAGCAGCAC
hKvLR2   ATTGGTGTCTCGTTCTTCGTTCTTCCTGCTGGCATTTTGGGATCGGGCTTTGCCCTGAAAGTCCAAGAGCAGCAT
rKvLR1   ATTGGTGTCTCTCATTCTTCGCTCTCTTCCTGCTGGCATTTTGGGATCCGGCTTTGCCCTGAAAGTCCAAGAGCAGCAT

Consensus -G-CA-AA-CACTTTGAGAA--GG-GGAA-CC-GC-GC-G--CT-AT-CAG-C-GCCTGGAG-T--TA-GC-AC-
hKvLR1   CGTCAGAGCACTTTGAGAAAAAGGAGGAAGCCAGTCGCTCATTCAGGCTGCCTGGGAGGTATTATGCTACC
mKvLR1   AGGCAGAAGCACTTTGAGAAGAGGCGGAAACCCGGCAGCTGGGTCTGCCTGGAGATTTTACGCCACC
hKvLR2   CGGCAAAAACACTTTGAGAAAACGGCGCGGAAACCCGCGGAAACCCTGCGGCAGGTCTGCCTGGGAGATTCTATGCTACT
rKvLR1   CGGCAAAAACACTTTGAGAAAACGGCGGAATCCTGCGGCAGGTCTCATCCAGTCTGCCTGCGGAGATTCTATGCTACT

```
Consensus    AACC-C----G-A--GACCTG-----C-----G
hKvLR2       AACCCCAACAGGATTGACCTGGTTGGCGACATG
             |||| |    | | |||||| |         |
hKvLR1       AACCTCTCGCGCACAGACCTGCACTCCACGTGG
             ||||||||  ||||| ||||||||||||||||||
mKvLR1       AACCTCTCACGCACCGACCTGCACTCCACGTGG
             ||||||||||||||||||||||||||||||||||
rKvLR1       AACCTCTCACGCACCGACCTGCACTCCACGTGG
```

FIG. 16D

S1   S2

```
Consensus  ------P-KRN*A-YR--Q---Y--LERPRGWA--YHA-VFL-V--CL-L-V--T-KEYE--S---L--LE---I--FG-

hKvLR2     KTPLSRPVKRNAKYRRIQTLIYDALERPRGWALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGA
                     ||| | ||    |  -   ||||||| |||| ||||| || ||  | | |||||  |    -  |  |   | || -
hKvLR1     GAGAGKPPPKRN*AFYRKLQNFLYNVLERPRGWAFIYHAYVFLLVFSCLVLSVFSTIKEYEKSSEGALYILEIVTIVVFGV
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
mKvLR1     GAGAGKPPPKRN*AFYRKLQNFLYNVLERPRGWAFIYHAYVFLLVFSCLVLSVFSTIKEYEKSSEGALYILEIVTIVVFGV
                                                                     ||||||||||||||||||||||||
rKvLR1                                                           KEYEKSSEGALYILEIVTIVVFGV
```

S3   S4

```
Consensus  E---RIWAAGCCCRY-GWRGRLKFARKP-C---DI-VLIAS--V-A-G-QGNV-ATS*LRSLRFLQILRM-RMDRRGGTWKLL

hKvLR2     EFALRIWAAGCCCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNVLATS*LRSLRFLQILRMLRMDRRGGTWKLL
               ||||||||||||| ||||||||||||| |  ||  ||||   | | | ||| ||| ||||||||||||| ||||||||||||
hKvLR1     EYFVRIWAAGCCCRYRGWRGRLKFARKPFCVIDIMVLIASIAVLAAGSQGNVFATSALRSLRFLQILRMIRMDRRGGTWKLL
           |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
mKvLR1     EYFVRIWAAGCCCRYRGWRGRLKFARKPFCVIDIMVLIASIAVLAAGSQGNVFATSALRSLRFLQILRMIRMDRRGGTWKLL
           |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
rKvLR1     EYFVRIWAAGCCCRYRGWRGRLKFARKPFCVIDIMVLIASIAVLAAGSQGNVFATSALRSLRFLQILRMIRMDRRGGTWKLL
```

FIG. 17A

S5
P

```
Consensus  GS---AHSKEL-TAWYIGFL-LIL-SFLVYL-EK**-E-D-*********F-TYADALWWGLITL-TIGYGDK-P-TW-GRL-

hKvLR2     GSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEFETYADALWWGLITTLATIGYGDKTPKTWEGRLI
           || |||||| ||||||||| ||| |||||| |          *****  *FDTYADALWWGLITLTTIGYGDKYPQTWNGRLL
hKvLR1     GSVVYAHSKELVTAWYIGFLCLILASFLVYLAEK**GENDH********FDTYADALWWGLITLTTIGYGDKYPQTWNGRLL
           ||||||||||||||||||||||||||||||||||  |||||        |||||||||||||||||||||||||||||||||
mKvLR1     GSVVYAHSKELVTAWYIGFLCLILASFLVYLAEK**GENDH********FDTYADALWWGLITLTTIGYGDKYPQTWNGRLL
           |||||||||||||||||||||||||||||||||   |||||        |||||||||||||||||||||||||||||||||
rKvLR1     GSVVYAHSKELVTAWYIGFLCLILASFLVYLAEK**GENDH********FDTYADALWWGLITLTTIGYGDKYPQTWNGRLL
```

S6

```
Consensus  AATF-LIGVSFFALPAGILGSG-ALKVQEQHRQKHFEKRR-PAA-LIQ-AWR-YATN--R-DL----

hKvLR2     AATFSLIGVSFFALPAGILGSGLALKVQEQHRQKHFEKRKPAAELIQAAWRYYATNPNRIDLVGDM
           |||| ||||||||||||||||| |||||||||||||||| ||| ||| ||| ||||  | ||
hKvLR1     AATFTLIGVSFFALPAGILGSGFALKVQEQHRQKHFEKRRNPAAGLIQSAWRFYATNLSRTDLHSTW
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
mKvLR1     AATFTLIGVSFFALPAGILGSGFALKVQEQHRQKHFEKRRNPAAGLIQSAWRFYATNLSRTDLHSTW
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
rKvLR1     AATFTLIGVSFFALPAGILGSGFALKVQEQHRQKHFEKRRNPAAGLIQSAWRFYATNLSRTDLHSTW
```

FIG. 17B

KCNQ3

0.2 µA

0.2 s
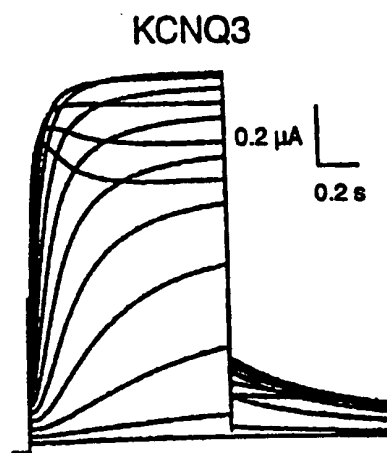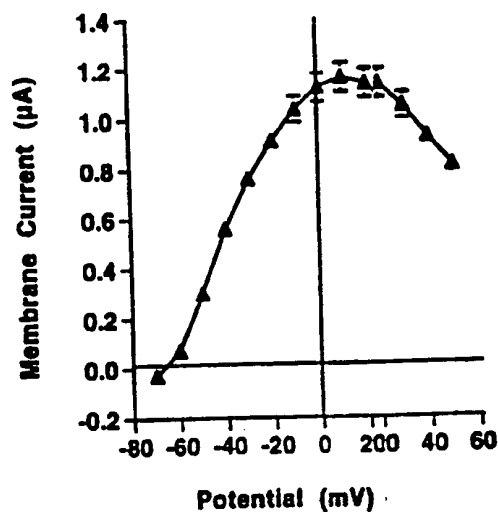
FIG. 18A

FIG. 18B

FIG. 18C
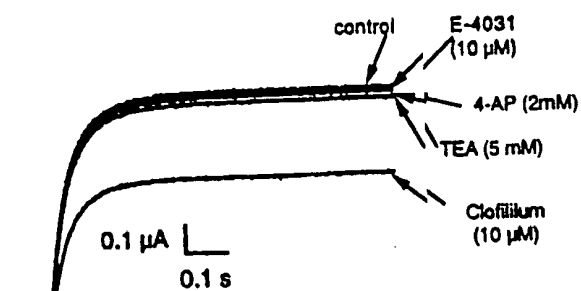
FIG. 18D)

KCNQ2   KCNQ3

FIG. 19A   FIG. 19B

KCNQ2+KCNQ3

2 µA

0.2 s
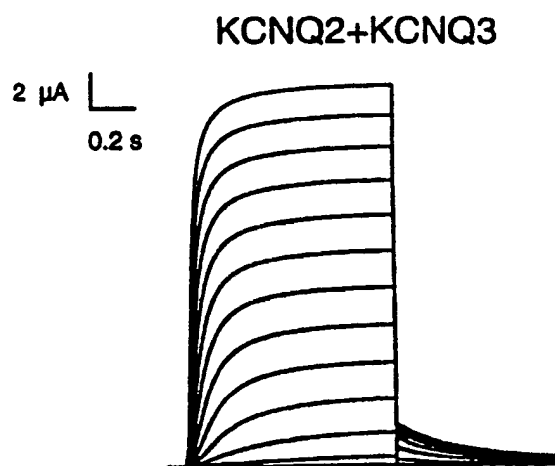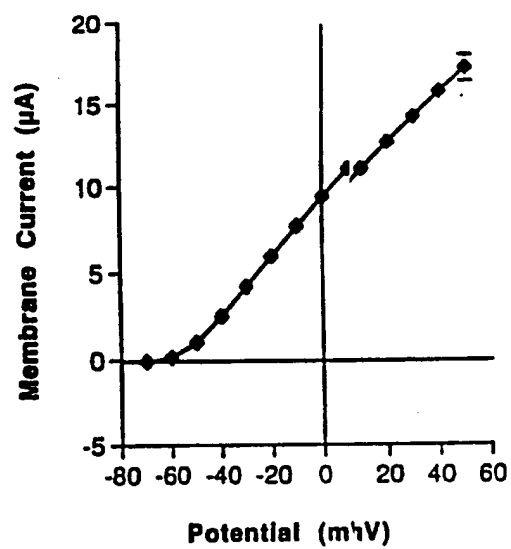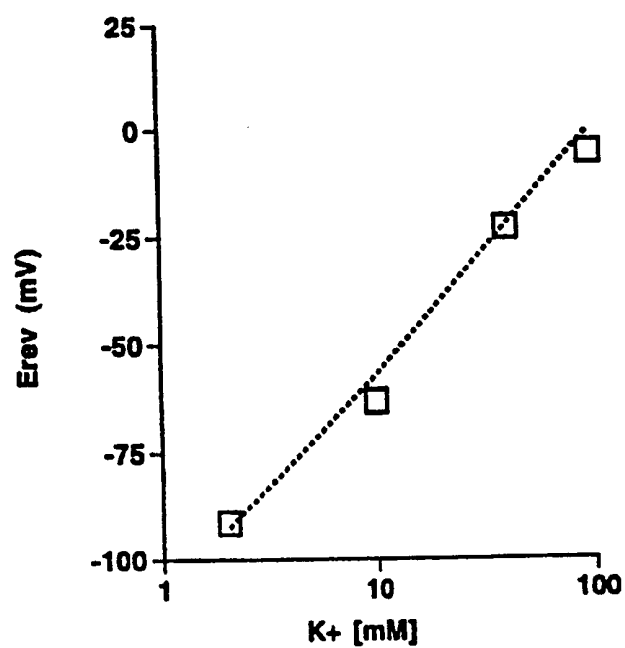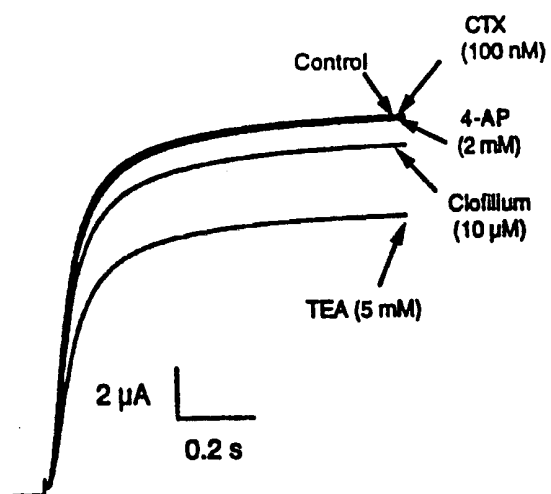
FIG. 19C

FIG. 19D
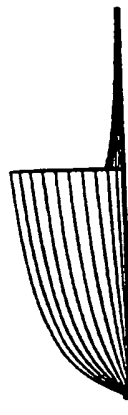
FIG. 19E

FIG. 19F

KCNE1

KCNQ2+KCNQ3

KCNQ2+KCNQ3
KCNE1

FIG. 20A

FIG. 20B

FIG. 20C

FIG. 21A

FIG. 21B

FIG. 22A

20 pA

100 mms
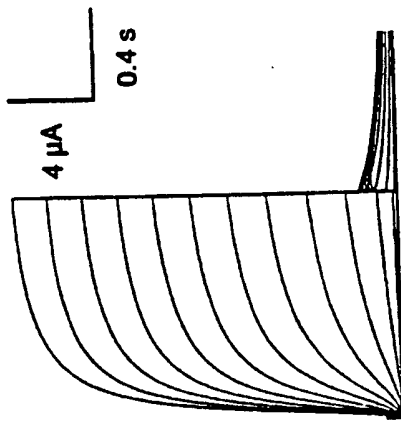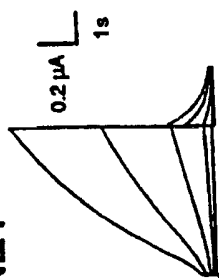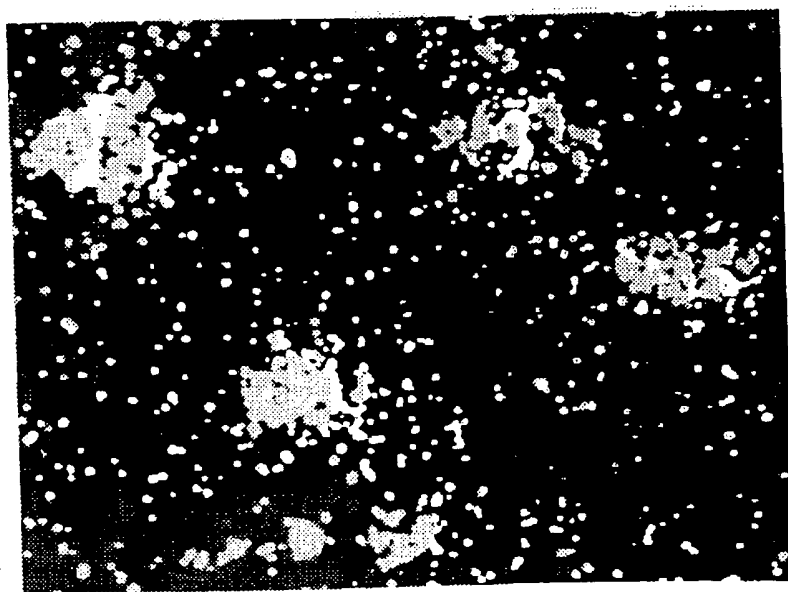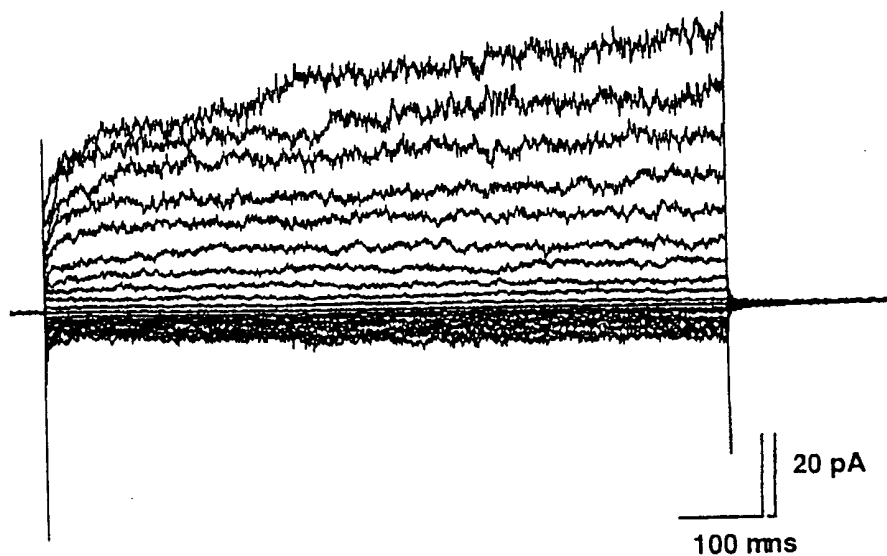
Open 2 ———
Open 1 ———
Closed - - - - -

5 pA

0.5 s
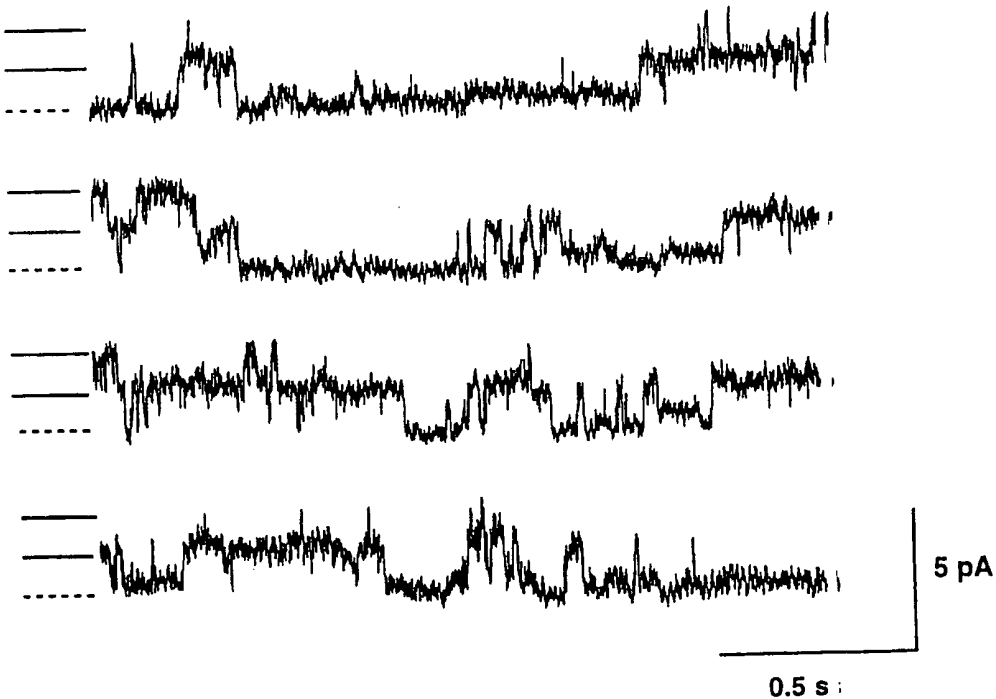
FIG. 22B

```
ATG GCA TTG GAG TTC CCG GCG GCT AAC CCA GCC GGA GGG GAC GCG 45
 M   A   L   E   F   P   A   A   N   P   A   G   G   D   A
                 5                  10                  15

GCG GCG GCC GGC GAC GAG GAG CGG AAA GTG GGG CTG GCG CCC GGC 90
 A   A   A   G   D   E   E   R   K   V   G   L   A   P   G
                20                  25                  30

GAC GTG GAG CAA GTC ACC TTG GCG CTC GGG GCC GGA GCC GAC AAA 135
 D   V   E   Q   V   T   L   A   L   G   A   G   A   D   K
                35                  40                  45

GAC GGG ACC CTG CTG CTG GAG GGC GGC GGC CGC GAC GAG GGG CAG 180
 D   G   T   L   L   L   E   G   G   G   R   D   E   G   Q
                50                  55                  60

CGG AGG ACC CCG CAG GGC ATC GGG CTC CTG GCC AAG ACC CCG CTG 225
 R   R   T   P   Q   G   I   G   L   L   A   K   T   P   L
                65                  70                  75

AGC CGC CCA GTC AAG AGA AAC AAC GCC AAG TAC CGG CGC ATC CAA 270
 S   R   P   V   K   R   N   N   A   K   Y   R   R   I   Q
                80                  85                  90

ACT TTG ATC TAC GAC GCC CTG GAG AGA CCG CGG GGC TGG GCG CTG 315
 T   L   I   Y   D   A   L   E   R   P   R   G   W   A   L
                95                 100                 105

CTT TAC CAC GCG TTG GTG TTC CTG ATT GTC CTG GGG TGC TTG ATT 360
 L   Y   H   A   L   V   F   L   I   V   L   G   C   L   I
               110                 115                 120

CTG GCT GTC CTG ACC ACA TTC AAG GAG TAT GAG ACT GTC TCG GGA 405
 L   A   V   L   T   T   F   K   E   Y   E   T   V   S   G
               125                 130                 135

GAC TGG CTT CTG TTA CTG GAG ACA TTT GCT ATT TTC ATC TTT GGA 450
 D   W   L   L   L   L   E   T   F   A   I   F   I   F   G
               140                 145                 150

GCC GAG TTT GCT TTG AGG ATC TGG GCT GCT GGA TGT TGC TGCC CGA 495
 A   E   F   A   L   R   I   W   A   A   G   C   C   C   R
               155                 160                 165

TAC AAA GGC TGG CGG GGC CGA CTG AAG TTT GCC AGG AAG CCCC CTG 540
 Y   K   G   W   R   G   R   L   K   F   A   R   K   P   L
               170                 175                 180

TGC ATG TTG GAC ATC TTT GTG CTG ATT GCC TCT GTG CCA GTGG GTT 585
 C   M   L   D   I   F   V   L   I   A   S   V   P   V   V
               185                 190                 195
```

FIG. 23A

```
GCT GTG GGA AAC CAA GGC AAT GTT CTG GCC ACC TCC CTG CGA  AGC 630
 A   V   G   N   Q   G   N   V   L   A   T   S   L   R    S
                200                 205                       210

CTG CGC TTC CTG CAG ATC CTG CGC ATG CTG CGG ATG GAC CGG  AGA 675
 L   R   F   L   Q   I   L   R   M   L   R   M   D   R    R
                215                 220                       225

GGT GGC ACC TGG AAG CTT CTG GGC TCA GCC ATC TGT GCC CAC  AGC 720
 G   G   T   W   K   L   L   G   S   A   I   C   A   H    S
                230                 235                       240

AAA GAA CTC ATC ACG GCC TGG TAC ATC GGT TTC CTG ACA CTC  ATC 765
 K   E   L   I   T   A   W   Y   I   G   F   L   T   L    I
                245                 250                       255

CTT TCT TCA TTT CTT GTC TAC CTG GTT GAG AAA GAC GTC CCA  GAG 810
 L   S   S   F   L   V   Y   L   V   E   K   D   V   P    E
                260                 265                       270

GTG GAT GCA CAA GGA GAG GAG ATG AAA GAG GAG TTT GAG ACC  TAT 855
 V   D   A   Q   G   E   E   M   K   E   E   F   E   T    Y
                275                 280                       285

GCA GAT GCC CTG TGG TGG GGC CTG ATC ACA CTG GCC ACC ATT  GGC 900
 A   D   A   L   W   W   G   L   I   T   L   A   T   I    G
                290                 295                       300

TAT GGA GAC AAG ACA CCC AAA ACG TGG GAA GGC CGT CTG ATT  GCC 945
 Y   G   D   K   T   P   K   T   W   E   G   R   L   I    A
                305                 310                       315

GCC ACC TTT TCC TTA ATT GGC GTC TCC TTT TTT GCC CTT CCAA GCG 990
 A   T   F   S   L   I   G   V   S   F   F   A   L   P    A
                320                 325                       330

GGC ATC CTG GGG TCC GGG CTG GCC CTC AAG GTG CAG GAG CAAA CAC 1035
 G   I   L   G   S   G   L   A   L   K   V   Q   E   Q    H
                335                 340                       345

CGT CAG AAG CAC TTT GAG AAA AGG AGG AAG CCA GCT GCT GAGG CTC 1080
 R   Q   K   H   F   E   K   R   R   K   P   A   A   E    L
                350                 355                       360

ATT CAG GCT GCC TGG AGG TAT TAT GCT ACC AAC CCC AAC AGGG ATT 1125
 I   Q   A   A   W   R   Y   Y   A   T   N   P   N   R    I
                365                 370                       375

GAC CTG GTG GCG ACA TGG AGA TTT TAT GAA TCA GTC GTC TCTT TTT 1170
 D   L   V   A   T   W   R   F   Y   E   S   V   V   S    F
                380                 385                       390
```

FIG. 23B

```
CCT TTC TTC AGG AAA GAA CAG CTG GAG GCA GCA TCC AGC CAA  AAG 1215
 P   F   F   R   K   E   Q   L   E   A   A   S   S   Q    K
                395                 400                 405

CTG GGT CTC TTG GAT CGG GTT CGC CTT TCT AAT CCT CGT GGT  AGC 1260
 L   G   L   L   D   R   V   R   L   S   N   P   R   G    S
                410                 415                 420

AAT ACT AAA GGA AAG CTA TTT ACC CCT CTG AAT GTA GAT GCC  ATA 1305
 N   T   K   G   K   L   F   T   P   L   N   V   D   A    I
                425                 430                 435

GAA GAA AGT CCT TCT AAA GAA CCA AAG CCT GTT GGC TTA AAC  AAT 1350
 E   E   S   P   S   K   E   P   K   P   V   G   L   N    N
                440                 445                 450

AAA GAG CGT TTC CGC ACG GCC TTC CGC ATG AAA GCC TAC GCT  TTC 1395
 K   E   R   F   R   T   A   F   R   M   K   A   Y   A    F
                455                 460                 465

TGG CAG AGT TCT GAA GAT GCC GGG ACA GGT GAC CCC ATG GCG  GAA 1440
 W   Q   S   S   E   D   A   G   T   G   D   P   M   A    E
                470                 475                 480

GAC AGG GGC TAT GGG AAT GAC TTC CCC ATC GAA GAC ATG ATC  CCC 1485
 D   R   G   Y   G   N   D   F   P   I   E   D   M   I    P
                485                 490                 495

ACC CTG AAG GCC GCC ATC CGA GCC GTC AGA ATT CTA CAA TTC  CGT 1530
 T   L   K   A   A   I   R   A   V   R   I   L   Q   F    R
                500                 505                 510

CTC TAT AAA AAA AAA TTC AAG GAG ACT TTG AGG CCT TAC GAT  GTG 1575
 L   Y   K   K   K   F   K   E   T   L   R   P   Y   D    V
                515                 520                 525

AAG GAT GTG ATT GAG CAG TAT TCT GCC GGG CAT CTC GAC ATG  CTT 1620
 K   D   V   I   E   Q   Y   S   A   G   H   L   D   M    L
                530                 535                 540

TCC AGG ATA AAG TAC CTT CAG ACG AGA ATA GAT ATG ATT TTC  ACC 1665
 S   R   I   K   Y   L   Q   T   R   I   D   M   I   F    T
                545                 550                 555

CCT GGA CCT CCC TCC ACG CCA AAA CAC AAG AAG TCT CAG AAAA GGG 1710
 P   G   P   P   S   T   P   K   H   K   K   S   Q   K    G
                560                 565                 570

TCA GCA TTC ACC TTC CCA TCC CAG CAA TCT CCC AGG AAT GAAA CCA 1755
 S   A   F   T   F   P   S   Q   Q   S   P   R   N   E    P
                575                 580                 585
```

FIG. 23C

```
TAT GTA GCC AGA CCA TCC ACA TCA GAA ATC GAA GAC CAA AGC ATG 1800
 Y   V   A   R   P   S   T   S   E   I   E   D   Q   S   M
                590             595                         600

ATG GGG AAG TTT GTA AAA GTT GAA AGA CAG GTT CAG GAC ATG GGG 1845
 M   G   K   F   V   K   V   E   R   Q   V   Q   D   M   G
                605             610                         615

AAG AAG CTG GAC TTC CTC GTG GAT ATG CAC ATG CAA CAC ATG GAA 1890
 K   K   L   D   F   L   V   D   M   H   M   Q   H   M   E
                620             625                         630

CGG TTG CAG GTG CAG GTC ACG GAG TAT TAC CCA ACC AAG GGC ACC 1935
 R   L   Q   V   Q   V   T   E   Y   Y   P   T   K   G   T
                635             640                         645

TCC TCG CCA GCT GAA GCA GAG AAG AAG GAG GAC AAC AGG TAT TCC 1980
 S   S   P   A   E   A   E   K   K   E   D   N   R   Y   S
                650             655                         660

GAT TTG AAA ACC ATC ATC TGC AAC TAT TCT GAG ACA GGC CCC CCG 2025
 D   L   K   T   I   I   C   N   Y   S   E   T   G   P   P
                665             670                         675

GAA CCA CCC TAC AGC TTC CAC CAG GTG ACC ATT GAC AAA GTC AGC 2070
 E   P   P   Y   S   F   H   Q   V   T   I   D   K   V   S
                680             685                         690

CCC TAT GGG TTT TTT GCA CAT GAC CCT GTG AAC CTG CCC CGA GGG 2115
 P   Y   G   F   F   A   H   D   P   V   N   L   P   R   G
                695             700                         705

GGA CCC AGT TCT GGA AAG GTT CAG GCA ACT CCT CCT TCC TCA GCA 2160
 G   P   S   S   G   K   V   Q   A   T   P   P   S   S   A
                710             715                         720

ACA ACG TAT GTG GAG AGG CCC ACG GTC CTG CCT ATC TTG ACT CTT 2205
 T   T   Y   V   E   R   P   T   V   L   P   I   L   T   L
                725             730                         735

CTC GAC TCC CGA GTG AGC TGC CAC TCC CAG GCT GAC CTG CAG GGC 2250
 L   D   S   R   V   S   C   H   S   Q   A   D   L   Q   G
                740             745                         750

CCC TAC TCG GAC CGA ATC TCC CCC CGG CAG AGA CGT AGC ATC ACG 2295
 P   Y   S   D   R   I   S   P   R   Q   R   R   S   I   T
                755             760                         765

CGA GAC AGT GAC ACA CCT CTG TCC CTG ATG TCG GTC AAC CAC GAG 2340
 R   D   S   D   T   P   L   S   L   M   S   V   N   H   E
                770             775                         780
```

FIG. 23D

```
GAG  CTG  GAG  AGG  TCT  CCA  AGT  GGC  TTC  AGC  ATC  TCC  CAG  GAC   AGA  2385
 E    L    E    R    S    P    S    G    F    S    I    S    Q    D     R
                     785                           790                 795

GAT  GAT  TAT  GTG  TTC  GGC  CCC  AAT  GGG  GGG  TCG  AGC  TGG  ATG   AGG  2430
 D    D    Y    V    F    G    P    N    G    G    S    S    W    M     R
                     800                           805                 810

GAG  AAG  CGG  TAC  CTC  GCC  GAG  GGT  GAG  ACG  GAC  ACA  GAC  ACG   GAC  2475
 E    K    R    Y    L    A    E    G    E    T    D    T    D    T     D
                     815                           820                 825

CCC  TTC  ACG  CCC  AGC  GGC  TCC  ATG  CCT  CTG  TCG  TCC  ACA  GGG   GAT  2520
 P    F    T    P    S    G    S    M    P    L    S    S    T    G     D
                     830                           835                 840

GGG  ATT  TCT  GAT  TCA  GTA  TGG  ACC  CCT  TCC  AAT  AAG  CCC  ATT   TAA  2565
 G    I    S    D    S    V    W    T    P    S    N    K    P    I     *
                     845                           850            854
```

FIG. 23E

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12N 5/10, 15/12, 15/63
US CL :435/320.1, 325; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/320.1, 325; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, searckch terms used)

APS, STN, BIOSCIENCE, CAPLUS, MEDLINE, BIOSIS, WPIDS

search terms: potassium channel, KCNQ, KvLQT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relelevant to claim No.
X	BARHANIN et al. K,LQT1 and Isk (minK) proteins associate to form the I_{ks} cardiac potassium current. Nature. 07 November 1996, Vol. 384, pages 78-80, especially page 78 and figure 1.	1-17
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A		19

☐ Further documents are listed in the continuation of Box C.	☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application b·but cited to understand the principle or theory underlying the inventioion
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimeded invention cannot be considered novel or cannot be considered to innvolve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimeded invention cannot be considered to involve an inventive step wlwhen the document is combined with one or more other such documennts, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search repport
29 JULY 1998	**81 AUG 1998**
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MICHAEL D. PAK
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International applicationn No.
PCT/US98/13276

Box I   Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheeiet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reeasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-17, 19
because they relate to parts of the international application that do not comply with the prescribed requirerements to such an extent that no meaningful international search can be carried out, specifically:

Claims 1-17 and 19 disclose nucleotide sequences. The search was carried to the extent possible without theoe nucleotide sequence listing.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of f Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report ccovers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did l not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international ssearch report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this internationnal search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-17, 19

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*